The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 11 APRIL, 1951 NUMBER 4
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LINEAR GROWTH RESPONSES OF BOYS TO PROLONGED TREATMENT WITH CHORIONIC GONADOTROPIN

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THE usefulness of chorionic gonadotropin[1] in treating certain types of sexual underdevelopment has been established by many investigations. Early studies (1) indicated that as genital development advanced under this treatment, growth and osseous development were accelerated. These findings were confirmed and extended in later investigations (2–14). However, the following aspects of the problem of growth responses to chorionic gonadotropin treatment remain unsettled: i) Are accelerated growth rates sustained as treatment is prolonged? ii) Are they maintained after it has been stopped? iii) At what age may the treatment be expected to stimulate the greatest growth? A statistical study of data pertinent to these questions is presented here.

METHODS

Forty-six boys, all but 1 of whom showed sexual underdevelopment,[2]

Received for publication September 28, 1950.

[1] Commercial chorionic gonadotropin has been used as follows: Follutein (E. R. Squibb & Sons), A. P. L. (Ayerst, McKenna & Harrison), Pranturon (Schering), and Pregnyl (Roche-Organon).

[2] This was evidenced by genital or gonadal hypoplasia, either alone or associated with cryptorchidism.

form the basis of this report. At the start of treatment with chorionic gonadotropin, all but 2 were undersized and they ranged in age from approximately 7 to 17 years. Periods of treatment varied from one to four and one-half years, but only 18 lasted two or more years. In most cases the treatments were uninterrupted. The effect of the treatment upon the patients' heights and growth has been the focus of this study. Twelve of the patients presented records permitting the study of their growth in the year before treatment. Eighteen patients returned more than six months after treatment for follow-up measurements. Their final heights, measured at intervals ranging from eight months to approximately eight years after treatment, and their post-treatment growth have been analyzed.

During treatment no attempt was made to maintain a constant dose of chorionic gonadotropin. Instead, the quantity was adjusted to the changing needs of the patient, as clinically appraised. Although this entailed varying the dose between 100 and 1,000 I.U. (administered three times a week), doses of 250 or 300 I.U. were the most frequent. In general, smaller quantities were given to younger patients and larger quantities to older ones. A few patients received some desiccated thyroid prior to, or at the beginning of, their treatment with chorionic gonadotropin.

In all cases roentgenograms of the skull, carpus and elbow were made just before treatment was initiated. Periodically, during treatment, others were made of the carpus and elbow, and the bone age was determined from the Shelton (15) standards. Passing attention has been given to the effect of chorionic gonadotropin upon the weight of 17 patients who, at the start of treatment, were underweight according to the Baldwin-Wood table (16).

Height (without shoes) was measured periodically during the course of treatment. Each boy's measurements were plotted on an individual Burgess (17) height chart (Fig. 1) on which the period of treatment was also marked. During the period of treatment these measurements were taken and plotted at extremely short intervals. Consequently, one may safely assume that the graph constructed from them shows the boy's height with reasonable accuracy at any age represented. When necessary, height readings from these charts have been used to supplement the actual measurements of the patient.

In studies of human growth, proper controls are a problem. Since both height and growth rate normally vary with age, neither can be evaluated accurately by comparison with that attained at a different age. Thus a boy's growth response to prolonged treatment cannot be evaluated by comparison with his own growth in the absence of treatment. Neither can one justify withholding treatment indefinitely from some patients merely to set up a group of controls identical in age with those receiving prolonged treatment.

In the present study each boy's 'control' was a measurement of the average height of boys the same age in years and months. The measurements of average height thus used were Burgess' measurements for the 50 per cent line[3] of her growth chart. They were obtained from an unpub-

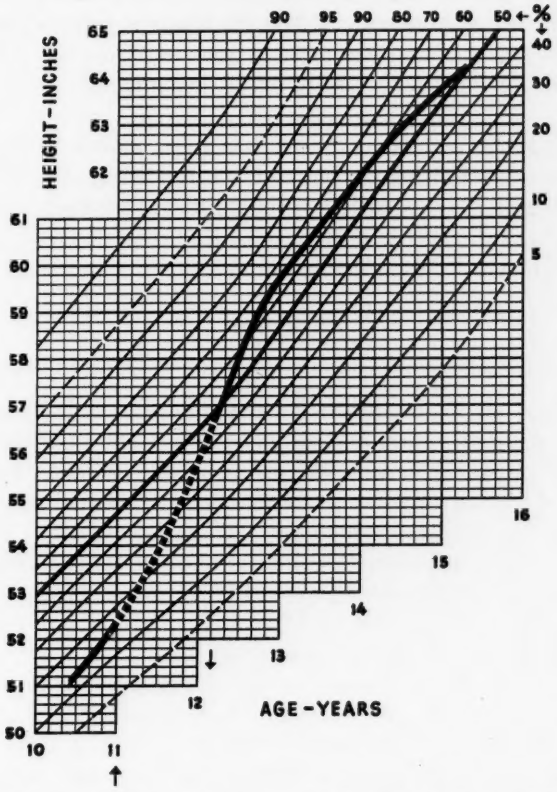

FIG. 1. Growth record of R. K. (Case 22) on Burgess height chart. Patient's height during treatment with chorionic gonadotropin is shown by broken line; heights before and after treatment are shown by heavy, solid lines. Numbers along the left side of the chart show height in inches; those to the right and below indicate age in years. Each Burgess curve shows the percentage (indicated by the number at the end of the curve) of boys as short as, or shorter than, the boy whose height falls on that curve. The 50 per cent curve is the standard of average height.

lished table generously contributed by Dr. Burgess, giving the height of the 50 per cent group at each month from birth to age 21. Using it, one

[3] If a boy's height falls upon this line at any age, it means that 50 per cent of American boys of that age are as short as or shorter than he is and 50 per cent are taller.

could compare each patient's height at any age with her standard of average height of boys of that age. Furthermore, the patient's growth in any desired interval of time could be calculated from his height data and compared with average growth similarly calculated from Burgess' table. Thus the heights and growth of all patients have been evaluated throughout by comparison with the same scale. Although Burgess' figures are a little low in comparison with some standards, it was felt that the advantages of following the above procedure fully offset any intrinsic limitations of the scale.

In analyzing the results, emphasis has been placed throughout upon group responses and either or both of the following statistical methods have been applied: (a) evaluation of the difference between two arithmetic means from the ratio of the difference to its probable error (18, 19), and (b) the Rank method (20).

RESULTS

Does chorionic gonadotropin stimulate acceleration of the growth rate?

The heights of all 46 patients at the beginning and end of treatment, and the average heights for boys of identical ages are shown with related data in Table 1. From these heights the means of the patients' heights (53.52 inches) and of the corresponding average heights (56.69 inches) at the beginning of treatment, were calculated. Comparison of these mean heights shows that the patients were 3.17 inches (5.6 per cent) below average at that point. Since this difference proved to be between three and four times its probable error, it is significant. Thus at the beginning of treatment, the group as a whole was definitely undersized.

A fuller concept of the composition of the group with regard to height at the start of treatment may be formed from Section A of Table 2. Here it will be seen that when treatment was begun, only 2 (4.3 per cent) of the patients were of average height or taller; that the others ranged in height from less than 1 inch to between 9 and 10 inches below average; and that patients with heights of 3 to 4 inches below average were the most frequent.

The means of the patients' heights (59.52 inches) and of the corresponding average heights (60.7 inches) at the close of treatment were calculated from the data in Table 1. Upon comparison, the patients' mean height was now only 1.18 inches (1.9 per cent) below the mean of the average heights. Since this difference was less than three times its probable error, it was of no significance. Thus, during chorionic gonadotropin treatment, the average height of the patients advanced from a level below average to one within the limits of average height.

The incidence of deviations of patients' heights from average heights

TABLE 1. HEIGHT AND GROWTH OF PATIENTS TREATED WITH CHORIONIC GONADOTROPIN COMPARED WITH AVERAGE MEASUREMENTS

Case number	Initials	Age of patient				Height at starting age (inches)		Height at closing age (inches)		Growth during treatment interval (inches)		Length of treatment	
		At start of treatment		At close of treatment									
		Yr.	Mo.	Yr.	Mo.	Patient	Average	Patient	Average	Patient	Average	Yr.	Mo.
1	S. R.	9	9	13	1	48.000	52.46	60.000	60.84	12.000	8.380	4	2
2	H. M.	13	7	14	7	57.500	60.01	62.250	62.52	4.750	2.510	1	0
3	G. B.	12	6	14	1	56.250	57.47	61.125	61.26	4.875	3.790	1	7
4*	L. A.	7	5	8	7	43.500	47.98			3.500	2.270	1	2
		9	7	12	5			56.000	57.29	6.700	5.130	2	10
5	B. L.	12	0	13	4	56.500	56.45	62.500	59.41	6.000	2.960	1	4
6	L. S.	8	10	13	4	45.000	50.73	55.750	59.41	10.750	8.680	4	6
7	T. C.	12	3	13	6	48.250	56.94	52.400	59.81	4.150	2.870	1	3
8	R. H.	7	3	8	11	45.000	47.64	50.600	50.89	5.600	3.250	1	8
9	J. C.	9	4	12	4	47.300	51.69	53.700	57.11	6.400	5.420	3	0
10	M. A.	14	11	17	0	53.500	63.32	60.000	66.71	6.500	3.390	2	1
11	E. O.	7	6	9	8	46.600	48.15	52.750	52.31	6.150	4.160	2	2
12	J. S.	8	9	10	2	50.250	50.57	54.250	52.91	4.000	2.340	1	5
13	R. A.	14	1	16	2	61.125	61.26	68.375	65.81	7.250	4.550	2	1
14	M. K.	13	1	14	6	55.125	58.81	62.250	62.31	7.125	3.500	1	5
15	P. H.	14	2	17	3	59.125	61.47	66.375	66.87	7.250	5.400	3	1
16*	J. M.	6	8	8	2	40.875	46.45			4.125	3.000	1	6
		9	8	10	3			49.500	53.36	2.250	1.05	0	7
17	H. M.	11	5	12	9	54.750	55.41	58.125	58.04	3.375	2.63	1	4
18	I. B.	13	9	14	11	60.500	60.42	65.250	63.32	4.750	2.90	1	2
19	R. S.	14	0	15	8	59.250	61.05	65.500	64.89	6.250	3.84	1	8
20	A. G.	14	6	16	0	56.750	62.31	61.500	65.52	4.750	3.21	1	6
21	R. B.	10	9	12	4	53.125	54.26	59.000	57.11	5.875	2.85	1	7
22	R. K.	11	0	12	2	52.375	54.71	56.500	56.77	4.125	2.06	1	2
23	J. M.	10	9	12	6	54.000	54.26	59.750	57.47	5.750	3.21	1	9
24	S. I.	8	6	10	1	47.250	50.09	53.250	53.06	6.000	2.97	1	7
25	A. B.	11	11	15	0	55.750	56.30	66.250	63.52	10.500	7.22	3	1
26	S. S.	16	4	17	6	59.125	66.06	63.750	66.99	4.625	0.93	1	2
27	J. S.	15	2	17	4	60.250	63.89	62.250	66.91	5.000	3.02	2	2
28	C. G.	14	7	16	0	57.875	62.52	63.125	65.52	5.250	3.00	1	5
29	M. D.	14	5	15	9	58.125	62.10	63.500	65.05	5.375	2.95	1	4
30	G. E.	14	0	16	8	59.000	61.05	65.500	66.44	6.500	5.39	2	8
31	A. P.	13	1	14	1	54.875	58.81	57.750	61.26	2.875	2.45	1	0
32	T. F.	13	0	14	2	57.250	58.61	61.750	61.47	4.500	2.86	1	2
33	S. C.	12	11	15	2	57.875	58.42	64.500	63.89	6.625	5.47	2	3
34	P. K.	13	8	15	0	56.250	60.21	61.250	63.52	5.000	3.31	1	4
35	J. M.	12	6	14	10	54.750	57.47	61.750	63.12	7.000	5.65	2	4
36	R. H.	13	8	14	11	59.500	60.21	63.625	63.32	4.125	3.11	1	3
37	A. S.	7	1	9	0	42.875	47.30	48.250	51.05	5.375	3.75	1	11
38	A. G.	10	0	11	1	49.000	52.91	53.000	54.85	4.000	1.94	1	1
39	A. S.	13	0	14	0	58.250	58.61	60.750	61.05	2.500	2.44	1	0
40	M. K.	15	5	17	0	61.375	64.40	65.500	66.71	4.125	2.31	1	7
41	R. R.	7	9	9	1	45.250	48.65	49.250	51.21	4.000	2.56	1	4
42	R. G.	9	3	11	10	47.750	51.53	53.625	56.15	5.875	4.62	2	7
43	H. G.	8	6	12	2	47.250	50.99	56.000	56.77	8.750	5.78	3	8
44	R. L.	12	4	14	5	53.500	57.11	61.875	62.10	8.375	4.99	2	1
45	R. W.	14	1	18	0	58.000	61.26	65.500	67.19	7.500	5.93	3	11
46	J. P.	16	2	18	2	56.125	65.81	59.600	67.23	3.475	1.42	2	0
Totals		(Cases 1 through 46)				2461.900	2608.13	2738.050	2790.32	271.600	177.42		
Means†		(Cases 1 through 46)				53.52	56.69	59.52	60.70	5.900	3.86		
Totals		(Cases 1 through 12)‡				597.650	643.41	684.925	710.41	81.375	55.15		
Means†		(Cases 1 through 12)‡				49.80	53.62	57.08	59.20	6.78	4.60		
Totals		(Cases 7 through 24)§						1049.075	1064.27				
Means†		(Cases 7 through 24)§						58.28	59.13				

* Two series of treatments were given. The actual growth during each period of treatment is shown, but height is given only at the beginning of the first series of treatments and at the end of the second series. Thus the difference between the initial and final heights shows the amount of growth between and during periods of treatment.

† All means are determined by dividing the totals by the number of persons involved.

‡ Growth records covering the year before treatment were available for these cases.

§ Growth records covering periods of more than six months after treatment were available for these cases.

at the close of treatment is shown in Section B of Table 2. Fourteen (30.4 per cent) of the patients were of average height or taller. The remainder ranged to no more than 8 inches below average, with heights of 1 inch or less under average being the most frequent. A comparison of patients'

TABLE 2. INCIDENCE OF DEVIATIONS OF PATIENTS' HEIGHTS FROM AVERAGE HEIGHT

Amount of deviation (inches)	+3* to +4	+2 to +3	+1 to +2	0 to +1	0 to −1†	−1 to −2	−2 to −3	−3 to −4	−4 to −5	−5 to −6	−6 to −7	−7 to −8	−8 to −9	−9 to −10
A. At the start of treatment														
Case numbers				#5	#12	#3	#2	#14	#1	#6	#26		#7	#10
				#18	#13	#11	#8	#27	#4	#16				#16
					#17	#19	#15	#29	#9	#20				
					#23	#21	#22	#31	#28					
					#25	#32	#24	#34	#37					
					#33		#30	#38						
					#36		#35	#40						
					#39			#41						
								#42						
								#43						
								#44						
								#45						
Totals				*2*	*8*	*5*	*7*	*12*	*5*	*3*	*1*	*0*	*1*	*2*
Percentage of 46 cases				4.3	17.4	10.9	15.2	26.0	10.9	6.5	2.2	0.0	2.2	4.3
B. At the close of treatment														
Case numbers	#5	#13	#12	#11	#1	#4	#28	#6	#20		#10	#7		
		#23	#18	#17	#2	#27	#34	#9				#46		
		#25	#21	#19	#3	#29	#37	#16						
				#24	#8	#35	#42	#26						
				#32	#14	#38		#31						
				#33	#15	#40								
				#36	#22	#41								
					#30	#45								
					#39									
					#43									
					#44									
Totals	*1*	*3*	*3*	*7*	*11*	*8*	*4*	*5*	*1*	*0*	*1*	*2*	*0*	*0*
Percentage of 46 cases	2.2	6.5	6.5	15.2	23.9	17.4	8.7	10.9	2.2	0.0	2.2	4.3	0.0	0.0
C. At the close of the follow-up periods														
Case numbers	#13		#12	#21	#15	#8	#20		#9		#10	#7		
			#17	#22	#19	#14	#23		#11					
			#18				#24		#16					
Totals	*1*	*0*	*3*	*2*	*2*	*2*	*3*	*0*	*3*	*0*	*1*	*1*	*0*	*0*
Percentage of 18 cases	5.5	0.0	16.6	11.1	11.1	11.1	16.6	0.0	16.6	0.0	5.5	5.5	0.0	0.0

* Plus deviations represent increases over average height.
† Minus deviations represent decreases from average height.

deviations from average height at the start and close of treatment (Sections A and B, Table 2) discloses their accelerated growth during treatment. This result, coupled with the advance in the patients' mean height shown above, amply demonstrates the stimulation of accelerated growth by chorionic gonadotropin.

To approximate the amount of growth acceleration effected in these 46 patients, the growth of each under treatment (Table 1) was calculated. From these increments the mean growth (5.9 inches) of the patients under treatment was computed. This mean, when compared with that of the

corresponding average growth increments (3.86 inches) which had been similarly determined, proved to be 2.04 inches (52.8 per cent) greater than the mean of the average increments. Since this difference is more than four times its probable error, it is of undoubted significance.

Final evidence of growth stimulation by chorionic gonadotropin was obtained from the 12 patients (Cases 1 through 12) whose growth in the year before treatment had been measured. A preliminary check by the Rank method showed these boys to be undersized at the start of treatment, and thus representative of the entire group of patients treated. Their growth in the year before treatment (Table 3) was then compared with

TABLE 3. GROWTH OF PATIENTS FOR ONE YEAR JUST PRECEDING TREATMENT, COMPARED WITH CORRESPONDING AVERAGE GROWTH

Case number	Initials	Range in age (Yr. mo.—Yr. mo.)	Patient's growth (inches)	Average growth (inches)
1	S. R.	8.9 – 9.9	2.00	1.89
2	H. M.	12.7 –13.7	1.60	2.35
3	G. B.	11.6 –12.6	1.05	1.97
4	L. A.	6.5 – 7.5	1.90	2.05
5	B. L.	11.0 –12.0	2.50	1.74
6	L. S.	7.10– 8.10	2.10	1.92
7	T. C.	11.3 –12.3	3.30	1.79
8	R. H.	6.3 – 7.3	1.90	2.07
9	J. C.	8.4 – 9.4	1.55	1.92
10	M. A.	13.11–14.11	1.30	2.48
11	E. O.	6.6 – 7.6	1.70	2.04
12	J. S.	7.9 – 8.9	2.25	1.92
Totals			*23.15*	*24.14*
Mean growth			*1.93*	*2.01*

corresponding average growth as follows: First the mean of the patients' growth (1.93 inches) and that (2.01 inches) of the corresponding average growth increments were calculated and compared. The patients' mean growth for that year was thereby shown to be only 0.08 inch (3.98 per cent) below average. Next the growth increments of the patients were compared with corresponding average growth increments by the Rank method, in order to evaluate the difference between them. This disclosed that in the year before treatment, the growth of the patients was not significantly different from average growth. We may thus conclude that at the start of treatment, these 12 patients, although undersized, were growing at the average rate.

How did chorionic gonadotropin affect the growth of these 12 boys? To determine this, the mean of their growth during treatment (Table 1, Cases 1 through 12) was now calculated and compared with the mean of the corresponding average growth increments. The mean for the patients was 6.78 inches as against a mean of 4.60 inches for the average growth increments—a gain of 2.18 inches or 47 per cent over average growth. Compared by the Rank method, the growth of these patients during treatment proved significantly greater than average growth. Here then is direct evidence of almost a 50 per cent increase in growth rate, stimulated by chorionic gonadotropin. Together with the relative increases in height and growth shown by all 46 patients during treatment, it gives conclusive proof that chorionic gonadotropin stimulates pronounced acceleration of linear growth in undersized, sexually underdeveloped boys.

Are accelerated growth rates sustained as treatment is prolonged?

Eighteen of the 46 patients were under treatment for two or more years. Their growth increments for each successive twelve months of treatment, and the deviation of each increment from corresponding average growth are shown in Table 4. Because growth rate varies with age, critical evidence of changes in growth acceleration in succeeding years could be obtained only by comparing the patients' deviations from average growth in those years. As seen from Table 4, the means of the patients' deviations from average growth in the first, second and third years of treatment were +1.179, +0.817 and −0.21 inches, respectively. Comparison of these means reveals a growth decline of only 0.362 inch between the first and second years of treatment; of 1.027 inches between the second and third years of treatment; and of 1.389 inches between the first and third years of treatment. Each of these differences was evaluated by comparison with its probable error. On this basis, only the difference between the average deviations for the first and third years of treatment, showed indisputable significance. A check of this evaluation by the Rank method confirmed this result. Apparently then, chorionic gonadotropin treatment may be continued for two years without measurable reduction of its growth-stimulating power. However, in a third year of treatment, this power declines distinctly.

Is the amount of growth attained during treatment with chorionic gonadotropin affected by the age of the patient?

The exercise of especial judgment was required in selecting the twelve-month periods for this growth data, since it was important to use an interval as close as possible both to the patient's birthday and to the begin-

ning of treatment. In the vast majority of cases it was possible to use a year-interval that came within three months of the patient's 'birthday year' and so justified comparison of the patient's growth therein with Burgess' average growth increments for successive 'birthday years.' The patients' increments of growth for these one-year intervals were tabulated according to age and the mean increment determined for each age represented. From these means a graph has been plotted (Fig. 2) showing the

TABLE 4. PATIENTS' GROWTH IN SUCCESSIVE YEARS OF TREATMENT, COMPARED WITH AVERAGE GROWTH*

Case No.	Initials	Starting age (Yr. mo.)	First year			Second year			Third year		
			Patient's growth (inches)	Average growth (inches)	Patient's growth minus average growth (inches)	Patient's growth (inches)	Average growth (inches)	Patient's growth minus average growth (inches)	Patient's growth (inches)	Average growth (inches)	Patient's growth minus average growth (inches)
1	S. R.	9.9	2.50	1.80	+0.7	2.9	1.14	+1.76	2.60	2.04	+0.56
4	L. A.	9.7	2.45	1.80	+0.65	3.65	1.74	+1.91	0.70	1.96	−1.26
6	L. S.	8.10	2.75	1.88	+0.87	4.35	1.80	+2.55	0.70	1.74	−1.04
9	J. C.	9.4	3.10	1.82	+1.28	2.20	1.38	+0.82	1.10	2.12	−1.02
10	M. A.	14.11	4.90	2.05	+2.85	1.60	1.28	+0.32			
11	E. O.	7.6	3.00	1.94	+1.06	2.8	1.92	+0.88			
13	R. A.	14.1	3.875	2.45	+1.425	3.20	1.96	+1.24			
15	P. H.	14.2	3.77	2.42	+1.35	2.00	1.92	+0.08	1.40	1.01	+0.39
25	A. B.	11.11	4.65	2.12	+2.53	3.60	2.42	+1.18	2.25	2.48	−0.23
27	J. S.	15.2	3.50	1.92	+1.58	1.50	1.01	+0.49			
30	G. E.	14.0	2.625	2.47	+0.155	3.125	2.00	+1.125			
33	S. C.	12.11	3.725	2.42	+1.30	2.10	2.48	−0.38			
35	J. M.	12.6	2.75	2.34	+0.41	2.50	2.50	±0.00			
42	R. G.	9.3	2.65	1.83	+0.82	1.60	1.77	−0.17			
43	H. G.	8.6	2.35	1.92	+0.43	2.40	1.80	+0.60			
44	R. L.	12.4	5.20	2.30	+2.9	3.00	2.48	+0.52			
45	R. W.	14.1	2.70	2.45	+0.25	2.40	1.96	+0.44	2.20	1.10	+1.10
46	J. P.	16.2	1.675	1.01	+0.665	1.80	0.41	+1.39			
Totals			*58.165*	*36.94*	*+21.225*	*46.725*	*31.97*	*+14.715*	*10.95*	*12.45*	*−1.5*
Means			*3.23*	*2.051*	*+ 1.179*	*2.595*	*1.776*	*+ 0.817*	*1.56*	*1.78*	*−0.21*

* In Cases 1, 6, and 45 the patients received four full years of treatment. In the fourth year, 2 of these patients showed further declines with respect to average growth, while the third did not. Since the data were insufficient for statistical evaluation, they have not been shown.

relation of age to the amount of growth attained in the first 'birthday year' of treatment. Average growth for the ages represented has been plotted for comparison. In view of the small number of cases involved at each age, the graph of the case material shows surprising smoothness, having only one minor fluctuation[4] at age 12 to 13. Still, one must recognize the likelihood of the curve's being altered at any one point by the

[4] Checked by the Rank method, the growth rate at this point was not significantly above that at adjacent points.

inclusion of additional cases. However, a statistical check showed (a) that the curve level maintained between the ages of 11 and 16 is significantly higher than that between the ages of 7 and 11, and also significantly above the level at age 16 to 17; and (b) that all points on the curve are significantly higher than normal growth levels for the same ages. Therefore, the following conclusions seem warranted: (i) chorionic gonadotropin may stimulate growth acceleration at any age, and (ii) the largest growth increments may be expected to result if the patient starts treatment when he is from 11 to 15 years old.

Do accelerated growth rates persist after therapy is discontinued?

To reduce the influence of responses to treatment in this portion of our data, only growth records covering periods of more than six months after

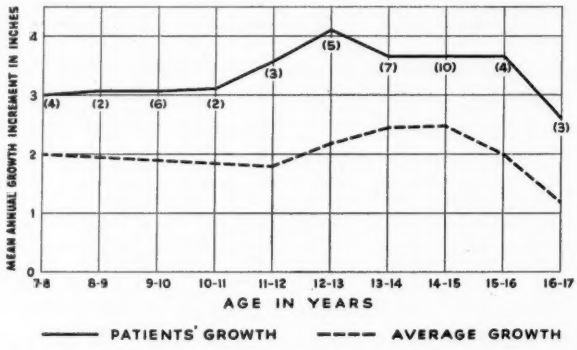

FIG. 2. Mean growth of patients in their first birthday-years of treatment compared with average annual growth increments. Age in years is shown along the abscissa and annual growth in inches is shown along the ordinate. Figures along the curve of patients' growth refer to the number of patients upon which each mean is based.

treatment, were analyzed. Eighteen patients (Cases 7 through 24) presented such data. Using the Rank method, a separate check was made of the height of these patients at the close of treatment (Table 1). They proved to be representative of the entire group of patients treated, in being within the limits of average height when treatment was stopped. Their mean height (58.28 inches) at that time was only 0.85 inch or 1.4 per cent below the mean (59.13 inches) of corresponding average heights.

The heights of these patients at the close of the follow-up period, were then considered (Table 5) along with corresponding average heights. When compared by the Rank method, these heights showed a difference (probability 0.05) which might, in the absence of contrary indications, be regarded as significant. However, an evaluation of the difference between

the mean height of the patients (63.15 inches) and that of the average heights (64.77 inches) failed to confirm the evaluation obtained with the Rank method. Although the difference between these mean heights (1.62 inches) showed the patients to be two and one-half per cent below average height, it proved to be decisively less than three times its probable error and consequently, of no possible significance. We must therefore conclude

TABLE 5. PATIENT'S HEIGHT AND GROWTH FOLLOWING TREATMENT, COMPARED WITH AVERAGE MEASUREMENTS

Case No.	Initials	At close of follow-up period			During follow-up period		
		Patient's age (Yr. mo.)	Patient's ht. (inches)	Average ht. (inches)	Patient's growth (inches)	Average growth (inches)	Length of follow-up period (Yr. mo.)
7	T. C.	16.0	58.000	65.52	5.600	5.71	2.6
8	R. H.	15.1	62.400	63.71	11.800	12.82	6.2
9	J. C.	13.0	54.250	58.61	0.550	1.50	0.8
10	M. A.	18.0	60.600	67.19	0.600	0.48	1.0
11	E. O.	18.0	63.000	67.19	10.250	14.88	8.4
12	J. S.	14.1	62.500	61.26	8.250	8.35	3.11
13	R. A.	17.2	70.125	66.82	1.750	1.01	1.0
14	M. K.	16.8	65.000	66.44	2.750	4.13	2.2
15	P. H.	18.0	66.600	67.19	0.225	0.32	0.9
16	J. M.	13.3	55.200	59.21	5.700	5.85	3.0
17	H. M.	18.0	69.000	67.19	10.875	9.15	5.3
18	I. B.	17.8	69.000	67.07	3.750	3.75	2.9
19	R. S.	16.4	66.000	66.06	0.500	1.17	0.8
20	A. G.	18.0	64.700	67.19	3.200	1.67	2.0
21	R. B.	16.6	66.750	66.27	7.750	9.16	4.2
22	R. K.	15.3	64.125	64.06	7.625	7.29	3.1
23	J. M.	16.1	63.125	65.67	3.375	8.20	3.7
24	S. I.	13.3	56.375	59.21	3.125	6.15	3.2
Totals			*1136.750*	*1165.86*	*87.675*	*101.59*	
Means			*63.15*	*64.77*	*4.87*	*5.64*	

that the mean height of these 18 patients remained within average limits after treatment.

The deviations from average height shown by these 18 patients at the close of the follow-up observations are presented in Section C of Table 2. By comparing these deviations with those shown at the end (Section B, Table 2) and beginning (Section A, Table 2) of treatment, one may follow the general course of the height changes in these patients. This discloses

that 2 patients (Cases 9 and 24), by the end of the follow-up period, had resumed the same height with respect to normal that they held at the beginning of treatment; and 2 (Cases 11 and 23) were even shorter in comparison with average height than they were at the beginning of treatment. On the other hand, 5 patients (Cases 8, 14, 16, 19 and 21) had, by the end of the follow-up period, lost only part of the gains made during treatment; 5 (Cases 7, 10, 12, 15 and 18) had lost none of the gains made during treatment; and 4 (Cases 13, 17, 20 and 22) had made still further gains. Thus final measurements, made more than six months after the last treatment, showed that 14 of these 18 patients (77.8 per cent) had maintained at least part of the gains with respect to average height that they had made during treatment.

The measured increments of growth made by these 18 patients after treatment (Table 5) were now compared (by the Rank method) with corresponding average growth. No significant difference between them was indicated. This result was checked by evaluating the difference between the mean of the patients' growth and that of the average growth increments. This difference (0.77 inch; 13.65 per cent) proved to be much less than three times its probable error, and thus confirmed the evaluation by the Rank method showing that the post-treatment growth of these patients was within average limits. It would appear then from these analyses of post-treatment height and growth data, that following treatment, growth tends to proceed at average rates.

DISCUSSION

The accelerated growth rate during treatment with chorionic gonadotropin demonstrated in this group of 46 sexually underdeveloped boys, is in line with the results of previous investigations, although the amount of the increase (52.8 per cent) is somewhat above the average increases previously reported (36.9 per cent by Lurie and Hertzman (10) and 12.9 per cent by Gordon and Fields (9)). However, the choice of normal standards, among other factors, must always be considered in a comparison of such results, and as already mentioned, the Burgess standard used in this study is sometimes regarded as low.

Of greater clinical interest is the answer this study gives to the question, "At what age should chorionic gonadotropin treatment be initiated to stimulate the greatest linear growth?" The literature provides no clear-cut answer to that question. Some investigations have shown greater growth responses in younger patients, whereas others have shown them in children aged 12 years or over. In the present study, recourse to individual growth charts prepared for each patient, made it possible to measure each boy's growth between consecutive birthdays. By averaging

the growth increments made by boys of the same age in only their first birthday-year of treatment, the relation of age to the result could be more clearly brought out. This procedure, not reported elsewhere, has disclosed a sharp grouping of the ages where maximal growth was obtained. This was between the ages of 11 and 16. The mean, annual increments of growth under treatment were not only uniform for each age within those limits, but were also significantly larger than those attained at either earlier or later ages. Thus, to obtain the maximal growth response, chorionic gonadotropin treatment of stunted, sexually underdeveloped boys should be started at ages 11 through 15.

It is of interest that the ages at which these sexually underdeveloped patients made their greatest growth response to chorionic gonadotropin, are also the ages closest to those at which puberty is normally established. Richey (21), studying the relation of puberty to height, found the age of maturity normally correlated with the amount of growth in early years. Dividing his subjects into three groups according to the age when puberty was established, he found that those who matured earliest showed the greatest growth and maintained a height advantage until the latest group to mature reached puberty. In his paper, he shows the normal, annual, growth increments at puberty and just following it, for each of these groups. Since all of these increments are well below the maximal increments determined in the present study, it would appear that something more than the normal growth spurt of the pubertal years is attained by stimulation with chorionic gonadotropin.

In treating undersized patients with chorionic gonadotropin, there frequently arises a question as to how long the treatment may be expected to stimulate accelerated growth. Previous investigators have differed on this point. Gordon and Fields (9) reported that as treatment was prolonged from six months to two years, percentage gains over normal growth increased, but that these failed to continue as treatment was extended for a third year. On the other hand, Lurie and Hertzman (10) concluded from a two-year study, that the length of treatment had no effect upon the results. Clearly, within that two-year period, they failed to find evidence of the increasing gains reported by Gordon and Fields.

The present results for the first two years of treatment likewise failed to support Gordon and Fields' conclusion that growth benefits increased as treatment was prolonged from six months to two years. To be sure, about half of the present patients who were treated for two years showed greater increases over normal growth in the second year. However, the remainder showed larger increases in the first year and the average gains of the entire group for each year were not significantly different. This result obviously parallels that obtained by Lurie and Hertzman in their

two-year study. However, the diminished growth responses in the third year of treatment that are reported here, preclude their conclusion that the length of treatment is without effect upon the amount of growth response. Rather do they confirm the results that Gordon and Fields obtained in the third year of treatment as well as the conclusion drawn from them. It is thus evident that Lurie and Hertzman failed to continue treatment long enough to obtain diminished growth responses, and that their conclusion about the growth effects of prolonged treatment was, accordingly, premature.

The present conclusion, that the growth benefits of chorionic gonadotropin treatment show no measurable difference in the first two years of treatment, but decline in the third year, obviously derives partial support from each of the above studies. Based upon thorough statistical analyses of a larger number of cases than were involved in these previous studies, this conclusion should be a reliable answer to the question of growth responses to prolonged chorionic gonadotropin treatment.

It is impossible to say whether or not the diminished responses to a third year of continuous treatment demonstrated here were caused by such antihormone formation as Leathem and Rakoff (22) found in experiments with sheep anterior pituitary extract and human chorionic gonadotropin, since no tests were made to determine this. However, in a previous study by Dorff (23), tests of serum taken from 17 patients after two to seventeen months of chorionic gonadotropin treatment showed no antihormones. Furthermore, in the present study, some of the patients failed to show maximal gains over normal growth until the second or third year of treatment. These facts taken together would suggest that possible antihormone formation was of no real consequence in establishing the present results.

It should be noted that accelerated linear growth was not the only benefit attending the advance of sexual development in the patients studied. As was to have been expected from the results of other studies (24, 25), many of these patients made substantial gains in weight as well. In the 17 underweight patients, these weight gains were conspicuous. However, available standards of normal weight were not sufficiently detailed to permit a reliable, statistical evaluation of the weight changes. The improvement in mental attitude that commonly accompanied the physical gains noted, must also be reckoned among the benefits of the treatment.

It should be emphasized that whenever treatment with chorionic gonadotropin is indicated, it must be judiciously applied. In all patients it must be adjusted and, if necessary, stopped, in order to avoid undue development of the genitals or gonads or excessive advance of bone age. Roentgenograms must be made periodically throughout the period of treatment, so that the change in bone age may be followed.

On the basis of the results presented here, the following schedule of treatment is suggested for undersized, sexually underdeveloped boys: If treatment seems desirable for a boy aged 7 or younger, doses of 100 to 200 I.U. two or three times a week for a period of eight to twelve months, are suggested. Since the risk of overstimulation is greatest in these youngest patients, every precaution must be taken to guard against it. With boys 11 to 14 years old, treatment may be continued for two years, if necessary, to stimulate puberal changes. Doses of 300 to 500 I. U. three times a week are suggested for this group. For boys aged 14 or older, doses of 500 to 1,000 I. U. three times a week for two years, may be necessary for desirable growth and genital development. The oldest boys may require the maximal dose, 1,000 I. U. Their treatment may be continued until the epiphyses are closed.

It must be recognized that although the patients presented here made distinct growth gains during chorionic gonadotropin treatment, it is impossible to say whether or not their ultimate height was increased. Since all but one of these boys were sexually underdeveloped, the present data likewise permit no conclusions as to whether similar gains may be stimulated in boys with apparently normal sexual development.

SUMMARY

1. The linear growth response of 46 sexually underdeveloped boys to prolonged chorionic gonadotropin treatment, started when they were from 7 to 17 years old, has been analyzed statistically. Burgess' determination of the average height of boys of the same age in years and months was used as each boy's 'control.'
2. Growth stimulation by chorionic gonadotropin was indicated as follows:
   (a) During treatment periods of from one to four and one-half years the group height was raised from a level significantly below average to one within average limits.
   (b) Twelve members of the group, measured for a year before treatment, showed only average growth then, but significantly greater-than-average growth during treatment.
3. Eighteen boys receiving continuous treatment for two or more years showed no measurable loss of growth benefits until the third year.
4. Boys 11 through 15 years old when treatment was started showed more growth the first year than did older or younger boys.
5. The following indicate that after treatment growth tends to proceed at average rates:
   (a) The mean final height of 18 patients, measured eight months to eight years after treatment, remained within average limits.

(b) Their mean post-treatment growth was not significantly below the mean of corresponding average growth increments.

6. Some substantial weight increases were observed, but not evaluated statistically.
7. A schedule for chorionic gonadotropin treatment of similar cases is suggested.
8. The data permit no conclusions as to whether ultimate heights were affected or similar responses might be expected in boys with normally developed genitals.

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DISTRIBUTION OF CHORIONIC GONADOTROPIN IN MOTHER AND FETUS AT VARIOUS STAGES OF PREGNANCY*

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INTRODUCTION

THE occurrence of gonadotropic pregnancy hormones in some mammals presents a perplexing problem. So far, these hormones have been found in two widely separated taxonomic groups, the equidae and the primates. In the mare, production is limited to about the first half of pregnancy; the concentration is highest between the forty-fifth and eightieth days, and the hormone disappears completely by about the one hundred and seventy-fifth day (1). The rhesus monkey gives a positive reaction to tests for gonadotropin during only one week, between the eighteenth and twenty-fifth days of gestation (2). In the chimpanzee, gonadotropin appears about the twenty-fifth day, reaches a peak around the forty-second day, and disappears between the hundredth and the hundred-and-thirtieth days of gestation (3, 4).

Man is unique in that gonadotropic hormone appears as early as ten days after fertilization (5) and that from here on, production is maintained throughout the duration of pregnancy. A peak is reached sometime between the thirtieth and sixtieth days (6, 7), but is maintained for only a short time. Gonadotropin, in decreasing amount, is still demonstrable in the urine and the blood of the mother during several days following parturition.

In view of the fact that in the majority of mammalian species, pregnancy takes a normal course without similar hormone production, one must admit that the significance of the particular situations in horses and in primates seems rather enigmatic. However, there exist certain relationships which may lead toward a functional interpretation. It is well known that ovariectomy or selective removal of the corpora lutea during early pregnancy is incompatible not only with implantation, but also with maintenance of pregnancy. In man, removal of the ovaries before the second month of pregnancy is followed by abortion, and in the pregnant mare, ovariectomy before the two-hundredth day is unfavorable for the continuation of pregnancy. With this information at hand, several investigators have under-

Received for publication September 5, 1950.

* This work was aided by the National Research Council, Medical Division, Committee for Research in Problems of Sex, through grants administered by Professor Emil Witschi.

taken experiments to study the effect of chorionic gonadotropin on the corpus luteum. Hisaw (8) found that in the monkey chorionic gonadotropin prolongs the functional phase of the corpus luteum for as long as fifteen days. Brown and Bradbury (9) reported that injections of chorionic gonadotropin prolong the activity of the corpus luteum in man for at least ten days. These results appear to indicate that the gonadotropic hormone of pregnancy may be instrumental in maintaining and prolonging the active life of the corpus luteum. Therefore it is an interesting fact that the species which produce large amounts of pregnancy hormones, *e. g.*, horse and man, have a low content of luteinizing hormone in the hypophysis, as compared with the follicle-stimulating hormone (10, 11). This seems the more significant as the action of the gonadotropic hormone of pregnancy in man is primarily luteinizing in character.

It thus appears that the gonadotropic pregnancy hormones become of importance for the maintenance of pregnancy in mammals with hypophyses that are deficient in LH production. There are also indications that these hormones exert some influence upon differentiation and development of the fetus, especially its genital organs. As a first step toward the analysis of the effects of human pregnancy hormones upon sexual differentiation of the fetus, this paper presents the results of an investigation on the distribution of chorionic gonadotropin in the tissues and the fluids of both the mother and the fetus.

MATERIALS AND METHODS

The maternal and fetal tissues and fluids used in this study were mostly obtained from therapeutic abortion and autopsy cases. Additional urine and blood samples were collected in the obstetrical division of the Department of Obstetrics and Gynecology of the University Hospital. The author is indebted to the staff of this department, and to its director, Dr. E. D. Plass, for their collaboration in making the collections.

Determinations on the concentration of gonadotropic hormone in fetal and maternal tissues were started at the eleventh week of pregnancy. Fluid samples were assayed directly, or after acetone or alcohol precipitation. Urine was precipitated and purified after the method of Heller and Chandler (12). All tissues were first cut into small pieces and then acetone-dried. For assaying, their powders were dissolved in distilled water. If the gonadotropic potency of a tissue was low, it was extracted with alcohols of low concentration, and the combined extracts precipitated by addition of 4 to 5 volumes of absolute alcohol.

Twenty-one-day old female rats, and eighteen-day-old female mice were used. The rats were obtained from two inbred laboratory stocks, and the mice from a Swiss strain. The animals were weighed before the first

injection, and those not reaching a standard weight were eliminated. To insure an even growth rate, litters were culled soon after birth and limited maximally to 7 animals.

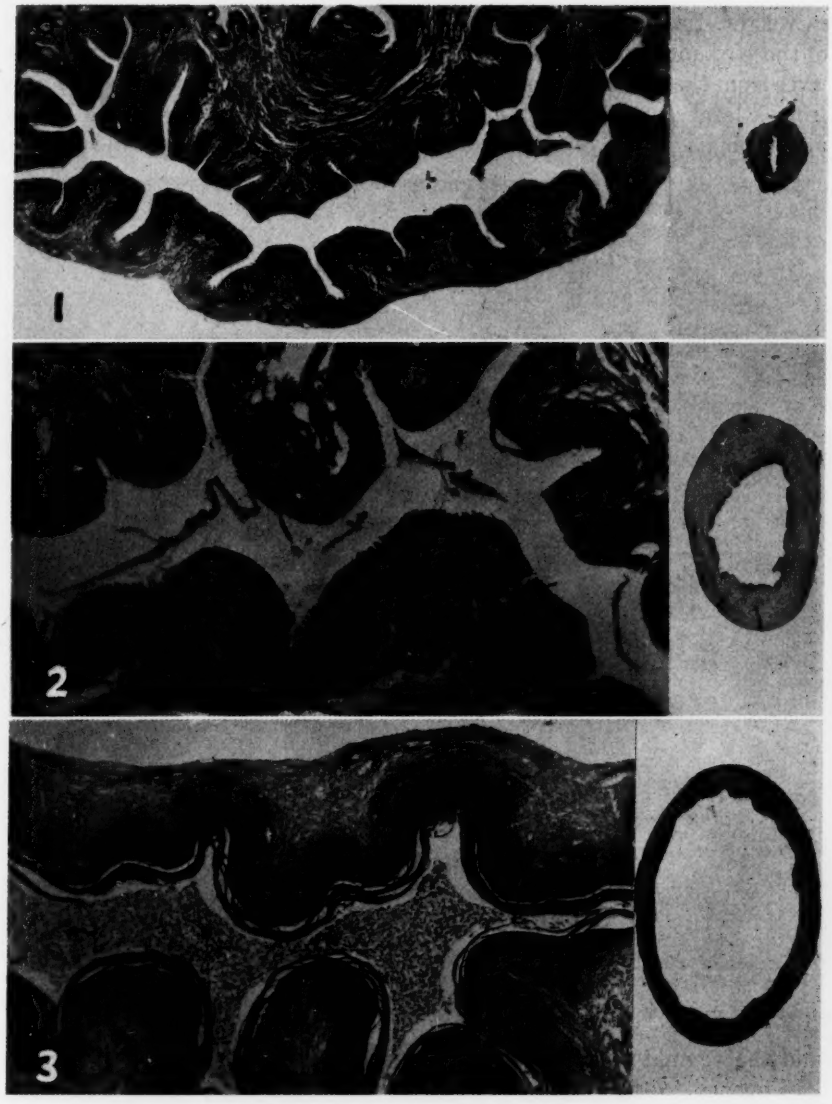
The assay material was administered in six subcutaneous injections at intervals of twelve hours. The animals were killed twenty-four hours after the last injection. The entire genital tract was dissected out, preserved in Bouin's fixative, and the gross effects recorded (Table 1). Sectioned tissues were dyed, preferably with Mallory's triple stain. All collected materials were assayed by the *serial method*, littermate females receiving graded quantities of the same sample. For instance, in one assay series of 4 rats, the littermates received 10 mg., 5 mg., and 1.25 mg. respectively. When all results proved to be positive, a series of 7 rats was next added, reaching

TABLE 1. ASSAY OF THE CHORIONIC PLACENTA OF THE FETUS TA 11 (ASSAY 46031)

Rat No.	Mg. of dried powder	Vagina		Uterus	Ovary	Text Fig.
		Cornified	Open			
1	5.0	+	+	Progestational	Ovulation	5
2	2.5	+	+	Estrous	Ovulation	—
3	1.25	+	+	Progestational	Ovulation	4
4	0.625	+	+	Estrous	Mature foll.	3
5	0.312	(+)	−	Proestrous	Enlarged foll.	2
6	0.156	−	−	—	—	—
7	0.078	−	−	—	—	1

into lower ranges (*cf.* Table 1). Assays near the critical level were repeated until the threshold value was fixed beyond question. For the present study, a total of 825 determinations with rats, and 296 with mice were made. Since about 50 different substances were assayed, this means that on an average, about 20 animals were used for each determination. Occasionally, 1 or 2 animals of a series were injected with an inactive protein, or left untreated; the serial method supplies further controls in the form of negative results *i.e.*, in females injected with subthreshold doses (Nos. 6 and 7, Table 1).

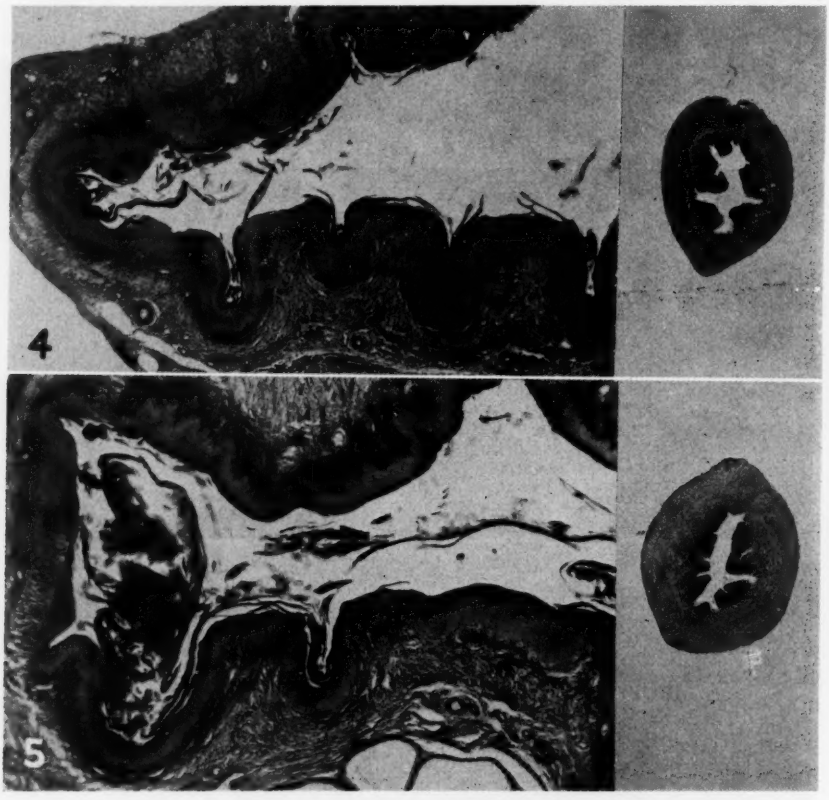
The criterion selected as an indicator of a positive reaction to gonadotropin, is the cornification of the vaginal epithelium. This is a clearly recognizable and reliable reaction. The cornification test takes into consideration only a single qualitative change. It is felt that it is superior to tests based on uterine enlargement, as this latter reaction is very complex, being composed not only of growth, but also of a series of functional and histologic changes. Methods based upon ovarian weight are considered even less adequate. Not only is there a considerable amount of variation in the



FIGS. 1–3. Cross-sections through vaginae (×50) and uteri (×15) of 5 rats of the assay series 46031. For explanations, *see* text, in last paragraphs of section on Materials and Methods; and Table 1.

the weight of the immature control ovaries, but the gonadotropins stimulate a great variety of reactions, some causing an increase, others a decrease

in weight. Immediately after ovulation, ovaries fall to nearly the weight of those of untreated littermates. The most serious objection, however, derives from the fact that the critical threshold value can be determined only from a large amount of material by statistical means. Nevertheless, due attention also was given to the changes that occur in the uteri and ovaries,



FIGS. 4–5. *See* legend of Figures 1–3.

and the vaginal orifice was routinely examined to determine if the canal had opened to the outside. All ovaries and representative parts of uterus and vagina were serially sectioned. The slides are kept on permanent file.

Figures 1 through 5 give the gamut of vaginal and uterine reactions obtained with injections of gonadotropin ranging from a subthreshold level up to that eliciting ovulation and luteinization of the ovary.

Figure 1 shows cross-sections through the typical immature vagina and uterus from a 25-day-old rat which had received a subthreshold dose of

chorionic placenta (Table 1, rat 7). They differ in no way from untreated controls.

Figure 2 shows a definite response, (Table 1, rat 5) and the initial stage of cornification of the vagina. The cuboid cells of the surface in the preceding stage are still attached to the wall, but beneath them are squamous and some cornified cells. The uterus is distinctly enlarged and the lumen somewhat distended.

Figure 3 shows a full estrous response (Table 1, rat 4) with a continuous but still attached cornified layer. In the lumen of the vagina, one notices an increased amount of mucus and sloughed off cuboid (nucleated) cells. The lumen of the vagina is greatly enlarged, and the uterus is maximally distended.

Figure 4 (Table 1, rat 3) shows a progestational response of the uterus, and full cornification of the vagina, with cells singly and in patches falling into the lumen.

Figure 5, shows the thickened progestational-type uterus. Masses of cornified cells lie in the lumen of the vagina, and the epithelium is transforming into a mucified state (Table 1, rat 1).

Parallel assays on rats and mice showed the mouse unit for vaginal cornification to be from four to four and one-half times smaller than the rat unit. Since, in the tables, all values had to be expressed in same kind, the mouse units were converted accordingly to rat units. The mouse was used as an assay animal whenever only relatively small amounts of material were available.

RESULTS

1. *Relative concentrations*

The condensed direct results of the assays are presented in Table 2. It shows the amount of dry powders and fluids that give an initial cornification of the vagina—the rat unit (R.U.). Whenever possible, the natural body fluids have been tested; but since assay rats were not available at all times, precipitates were collected and stored in the form of dry powders. Some weights of such powders containing 1 R.U. of gonadotropic principle are also included in Table 2.

In Table 3, the values for dry powders are calculated to give the number of R.U. per gram. This does not yet furnish a good picture of the relative concentrations in tissues and fluids; therefore the dry powder values were converted into R.U. per gram of fresh tissue. Since the weights of the fresh tissues had been taken in only a few cases, these values in Table 4 are summary approximations, based on the assumption that dry powders represent in weight: 10 per cent of fresh embryonic tissues, chorion, and uterine endometrium; 20 per cent of uterine muscle and of hypophysis;

and 25 per cent of long muscle of the maternal thigh. They are sufficiently accurate to permit direct comparisons with the R.U. per ml. values of the various tested fluids in the same table.

The study of these tables, particularly Table 4, reveals significant differences in concentration between the various tissues and fluids, at every stage. The chorion, which usually is assumed to produce the gonadotropic

TABLE 2. STANDARDIZATION ASSAYS OF NATURAL FLUIDS, DRY POWDERS OF TISSUES, AND PRECIPITATED BODY FLUIDS. AVERAGE AMOUNTS, IN MG. OR IN ML., PRODUCING INITIAL CORNIFICATION (RAT UNIT)

Case	Age, weeks	Length, mm.	*Fetus*				*Mother*				
			Body, mg.	Amniotic fluid		Chorion, mg.	Endometrium, mg.	Body, mg.	Blood		Urine (natural), ml.
				Natural, ml.	Dry, mg.				Natural, ml.	Dry, mg.	
VPW	7								0.002		
TA 11	9	37							0.005		
VPW	10								0.01		
TA 1	11	56	80	0.04*	0.22*	0.2	2	U:10	0.01	1.5	0.1
TA 8	11	60		0.09*	0.4 *	0.5			0.016		
TA 5	12	60	100	0.1 *	0.5 *	1.0	5		0.03		
HF 1	12		150					T, H:30	0.05		0.3
TA 2	14	95	500† L:125			2.5			0.1	19	
TA 9	15	115							0.075		
TA 10	16	122				2.5			0.1	19	
TA 7	18	140				2.5			0.1		
TA 3	21	180	800	0.4	3.8	4.5			0.2	36	0.5
HF 2	30	220				5.0					
HF 3	38	320	L:2500				75				
Caes. 4 days p.p.	40			1.2							3.0

* Mixture of amniotic and chorionic fluids.
† 400 mg. dry body tissue gave initial reaction.
H: Hypophysis. L: Liver. T: Muscle of thigh. U: Muscle of uterus. Caes.: Caesarean operation. p.p.: post partum

hormone, shows the highest concentration; then follows the maternal blood; and the endometrium is somewhat higher in hormone content than the rest of the maternal body tissues. It is obvious that all tissues are permeated with gonadotropin.

The body of the fetus as well as the fetal fluids contain smaller concentrations. The separately collected chorionic fluid of fetus TA 8 (11 weeks) had a higher gonadotropic content than its amniotic fluid (20, as against 10 R.U. per ml.). This might conceivably be an effect due to secondary concentration, though a more probable explanation will be proposed subsequently. The fresh chorionic fluid had an intense yellow-green color and was of gelatinous consistency. The amniotic fluid of the same fetus was a clear, only slightly yellowish, and freely-flowing liquid. The differ-

ence in consistency between these two fluids is reflected by the more copious precipitate obtained in the chorionic fluid. One ml. of the latter yielded 12 mg. of dry powder, whereas the same amount of amniotic fluid gave only 3.5 mg. of dry powder. In all, 43 ml. of amniotic fluid and 11 ml. of chorionic fluid were collected; in both instances this probably represents nearly 90 per cent of the total volume. In TA 1 and TA 5, the chorionic and the amniotic fluids were not separately collected. After the twentieth

TABLE 3. GONADOTROPIN CONTENT OF DRY POWDERS, IN RAT UNITS PER GRAM

Case	Age, weeks	Length, mm.	*Fetus*			*Mother*			
			Body, R.U./Gm.	Amniotic fluid, R.U./Gm.	Chorion, R.U./Gm.	Endometrium, R.U./Gm.	Body, R.U./Gm.		Blood, R.U./Gm.
VPW	7								
TA 11	9	37							
VPW	10								
TA 1	11	56	12.5	4500*	5000	500	U:	100	666
TA 8	11	60		2500*	2000				
TA 5	12	60	10	2000*	1000	200			
HF 1	12		7				T, H:	33	
TA 2	14	95	2		400				53
			L: 8						
TA 9	15	115							
TA 10	16	122			400				53
TA 7	18	140			400				
TA 3	21	180	1.25	263	222				28
HF 2	30	220			200				
HF 3	38	320	L: 0.4			13			

* Mixture of amniotic and chorionic fluids.
H: Hypophysis. L: Liver, T: Muscle of thigh. U: Muscle of uterus.

week, the amnion fuses with the chorion, so that the amniotic fluid fills the entire chorionic vesicle.

The body of the fetus contains only relatively small amounts of chorionic gonadotropin. As a consequence, several attempted assays gave negative results because insufficient amounts of tissue had been collected. Valid results were obtained for 6 fetuses (Tables 2–4). In the case of TA 1, the content per gram was sixteen times lower in the fetal than in the maternal body tissues.

In HF 1, the ratio of hormone concentrations between maternal somatic

muscle and trunk tissue of the fetus is 10:1. The other assays suggest that this ratio is probably characteristic for all later stages of pregnancy. The assay of fetal livers discloses concentrations higher than those in trunk tissues. On the other hand, fetal brain did not give a positive gonadotropic reaction, indicating a content lower than the average for fetal tissues.

TABLE 4. GONADOTROPIN CONTENT OF FRESH TISSUES IN RAT UNITS PER GRAM, AND OF NATURAL BODY FLUIDS IN RAT UNITS PER MILLIMETER

Case	Age, weeks	Length, mm.	*Fetus*			*Mother*			
			Body, R.U./Gm.	Amniotic fluid, R.U./ml.	Chorion, RU/Gm.	Endometrium, R.U./Gm.	Body, R.U./Gm.	Blood, R.U./Gm.	Urine, R.U./ml.
VPW	7							500	
TA 11	9	37						200	
VPW	10							100	
TA 1	11	56	1.25	25*	500	50	U:20	100	10
TA 8	11	60		12*	200			70	
TA 5	12	60	1.0	10*	100	20		33	
HF 1	12		0.7				T:8		
							H:7	20	3
TA 2	14	95	0.2		40			10	
			L:0.8						
TA 9	15	115						13	
TA 10	16	122			40			10	
TA 7	18	140			40			10	
TA 3	21	180	0.125	2.5	22			5	2
HF 2	30	220			20				
HF 3	38	320	L:0.04			1.3			
Caes.	40			0.8					
4d. p.p.									0.3

* Mixture of amniotic and chorionic fluids.
H: Hypophysis. L: Liver. T: Muscle of thigh. U: Muscle of uterus. p.p.: post partum.

2. *Temporal changes*

Tables 2–4 exhibit a continual decline of gonadotropic content in the fetal as well as in the maternal fluids and tissues. The value for the concentration in blood during the seventh week is probably still the maximum, which is generally reached sometime during the fifth, sixth or seventh weeks. A rapid fall is noted from the seventh to the eleventh week, and then a more gradual decline until the thirtieth week. Undoubtedly individual variations occur, but a much greater amount of material would be needed for their study. In the urine, the gonadotropic concentration falls more slowly than in the blood, and in general cannot be considered equally important for the present study, which is mainly concerned with a comparison of maternal and fetal milieus. From the collected data (Table 4) one may draw the general conclusion, that concentrations fall off similarly in all maternal and fetal tissues and fluids, except urine, and that therefore the proportions of relative concentrations remain the same. From the

eleventh week until parturition, the concentrations decrease to about one twenty-fifth.

3. *Termination of pregnancy*

The disappearance of gonadotropin in the urine and in the blood after delivery and after operative removal of the fetus, was followed in several cases. In TA 11, pregnancy was terminated at 9 weeks. One day after the operation there was a 10-fold decrease, and at two days, a further 5-fold decrease, *i.e.*, within two days the concentration of blood gonadotropin fell to one-tenth and finally one-fiftieth of the original level. In another pregnancy, terminated at 12 weeks (TA 5), there was a 50-fold decrease in blood gonadotropin within one day. In a third case terminated at 21 weeks (TA 3), there was a 10-fold decrease in urinary gonadotropin at four days and a further 12.5-fold decrease at six days postoperatively. Four days following the termination of a normal pregnancy, 3 ml. of urine were required to give a positive gonadotropic response. It is obvious that the blood values fall faster than those of urine. These data suggest that after therapeutic abortions the maternal system loses its large quantities of stored chorionic gonadotropin within the same time as after a normal delivery at term.

4. *Hypophyseal reactions*

The hypophysis is of particular interest in Case HF 1. Its value for total gonadotropic content is identical with that of the muscle of the thigh. In both instances, 30 mg. of dry powder correspond to 1 rat unit. Witschi and Riley (13) found 20 mg. of powder equivalent to 1 R.U. in two other pregnant women, and in a fourth case, "over 20 mg." The last one was a three-months' pregnancy, like that of HF 1. It would appear from such data that, during pregnancy, the human hypophysis ceases to produce its own gonad-stimulating hormones, and that it contains only the chorionic gonadotropic principle which is found in all maternal tissues.

In Case TA 1, a pregnancy of 11 weeks, the entire gravid uterus had been removed. Six days later, the patient suffered a cerebral hemorrhage and died. The hypophysis had, at this time, already regained its normal functional state (5 mg. of dry powder per R.U.).

DISCUSSION

a. *Relative concentration in maternal blood and urine*

The classic investigations of Aschheim and Zondek (14) showed that pregnancy urine is a rich source of gonadotropic hormone. The literature since 1927 is replete with studies on the quantitative variations of urinary

gonadotropins throughout pregnancy; but much less attention has been directed toward conditions in the maternal blood. Among the best known and most extensive studies on urinary gonadotropins are those by Browne and Venning (6) and by Evans, Kohls, and Wonder (7). The latter have also made some determinations of gonadotropin in blood. The present investigation shows that, although both urinary and blood values follow a common trend in falling off from the ninth week until delivery, the concentrations do not, in a strict sense, run parallel (Table 4). At 11 weeks (TA 1) the ratio of blood serum to urinary gonadotropin is of the order of 10:1; at 12 weeks (HF 1) it is 6.6:1; and at 21 weeks (TA 3) it has fallen to 2.5:1. On the other hand, there is a fairly constant ratio (4:1) between gonadotropic concentration at the site of its production (chorion) and in the maternal blood, at least from the eleventh through the twenty-first weeks of pregnancy. Obviously, changes in concentration of gonadotropin in the urine are not dependent exclusively upon the rate of production, and renal excretion does not seem to be the only, or even the most important means, of elimination of this hormone. This relationship is also suggested by the recent studies of Johnson, Albert, and Wilson (15), on the postpartum fate of the chorionic gonadotropin contained in mothers at the time of delivery. Careful measurements of the renal clearance of chorionic gonadotropin show that only 5.8 per cent appears later in active form in the urine. Since the present study proves that the tissues as well as the blood contain the hormone, it follows that less than 2 per cent of the total remaining gonadotropin leaves the body through urinary excretion.

The reported conditions lead to the following conclusions: 1) the concentration of gonadotropin in the blood serum is a more accurate expression of conditions in the maternal body, than the concentration in the urine; 2) the methods of gonadotropin elimination change during the progress of pregnancy; and 3) urinary excretion of gonadotropin becomes relatively more important during the later part of pregnancy. The present study is therefore not in agreement with the conclusions of Gastineau, Albert, and Randall (16) that changes in urinary excretion are direct reflections of changes in serum level, and therefore would depend only on changes in the rate of hormone production.

b. *Chorionic origin*

A large volume of evidence renders almost certain proof that the chorion is the site of gonadotropin production during pregnancy. The history of this question and, in general, our knowledge and interpretation of the chorionic hormones is completely reviewed by Courrier (17). The chorion was early credited with endocrine functions; Jones, Gey, and Gey

(18) have added experimental support to this surmise through studies on human chorionic cells *in vitro*. After the explant had been cultured long enough to consist entirely of new cells, and the media had been changed several times, positive gonadotropic responses still were obtained from this material. Everson (19), who employed cytochemical methods for identification of polysaccharides, believes that the stained granules in the Langhans cells of the chorionic villi (cytotrophoblast) represent the chorionic gonadotropin at the site of its production.

c. *Ratio between maternal and fetal concentrations*

As previously stated, the relative concentrations of the gonadotropic hormone in maternal and fetal body tissues maintain a proportion of about 10:1. It is believed to remain fairly constant throughout pregnancy. Only a few reports, dealing casually with the content of gonadotropin in the fetal body, have appeared in the literature. Geist and Spielman (20) mention that the equivalent of 54 Gm. of fetal liver gave a negative gonadotropic response. It is, however, difficult to judge the importance of this determination; the fetus was "dying in utero" and its age was not given, though possibly it was near term. In the present investigation the liver of TA 2, a male fetus 14 weeks old, showed a positive gonadotropic action at 125 mg. of dry powder, whereas 400 mg. of dried body tissue did not reach the threshold value. In 1940, Parker and Tenny (21) reported gonadotropin assays with maternal and fetal tissues from a series of 10 cases ranging from 4 to 8½ months. These observers came to the conclusion that normally gonadotropin is present in placenta, and in maternal blood and urine in equal amounts, but not in fetal organs, fetal blood or amniotic fluid. Uterine wall was the only maternal tissue they found to contain gonadotropin. Examination of their data leaves one with the impression that the quantities of maternal blood and urine and of placental tissues used for their assays were far too large to give characteristic data as to quantitative variations of the gonadotropic content. On the other hand, the amounts of fetal tissues were too small to demonstrate the gonadotropin that is actually present.

Toward the time of delivery, the gonadotropic output seems somewhat increased. However, data published by Sklow (22) and by Stroink and Mühlbock (23) show that the proportion between fetal (umbilical) and material blood concentrations remains between 1:10 and 1:20.

d. *Exchange between mother and fetus*

The data collected in this investigation and the conclusions so far drawn indicate that chorionic gonadotropin is produced by the fetus to create, in the body of the mother, favorable environmental conditions for its

own development. In other words, the hormone is produced to be released into the body of the mother, and not for use within the fetus. Seepage through the chorionic and amniotic membranes into the fetal fluids is not however effectively prevented, and some hormone also penetrates the body of the fetus. In the body, the hormone is kept at its low level, probably by washing out through the fetal blood stream and excretion through the placenta into the maternal circulation. Other possible methods of reduction of the hormone might involve its destruction or inactivation within the fetal body.

If on the contrary, one should consider the possibility of the hormone entering the body with the umbilical circulation, one would have to assume that the fetal kidneys, even at the age of 11 weeks, were able to excrete and flush it into the amniotic cavity. However, as is well known, the kidneys develop slowly and remain functionally inefficient until late in pregnancy. The relatively high concentration in the amniotic fluid—ten to twenty times that of body tissues—can therefore hardly be a result of renal elimination.

Our assays show that the concentration in the amniotic and chorionic fluids is only about half that of the enveloping maternal endometrium (Table 4). Such a condition favors the contention that the hormone enters the chorionic fluid from the uterine endometrium, probably across the chorion leve. Since the fetus is bathed by amniotic fluid, and also swallows it at quite early stages, it would seem probable that the relatively small amounts of the hormone in the fetal tissues are derived from the amniotic source, and not from the placenta by the umbilical vein. Experiments with tagged materials, like those of Flexner *et al.* (24) with radioactive sodium, may in the future provide more definite solutions to the problems of hormone transfer.

e. *Physiologic importance for fetal development*

Even though relatively low, the amount of gonadotropic hormone in the fetal body is physiologically significant. A fetus which at 11 to 12 weeks rates 1 rat unit per gram of fresh tissue, and which weighs about 30 grams, contains not less than thirty times the threshold dose, which in immature female rats of a similar size produces vaginal cornification and other characteristic reactions. Obviously, this is a very considerable concentration, and the possibility of its exerting an effect upon the fetal sex organs calls for a reinvestigation of this chapter of human embryology.

SUMMARY

1. The distribution and concentration of chorionic gonadotropin in human pregnancy have been determined by bioassay of systematically collected maternal and fetal tissues and fluids.

2. In all stages of pregnancy, the concentration is highest in the chorionic placenta, supporting the view that the hormone is produced by the embryonic trophoblast.

3. In the maternal body, the highest gonadotropic concentration is in the blood; and the uterine endometrium has a higher content than the other maternal tissues.

4. Concentration in the urine is not as adequate an expression of hormone production as concentration in the serum.

5. The body of the fetus contains only relatively small amounts of chorionic gonadotropin. The concentrations are of such a range, however, that they may be expected to be of physiologic significance.

6. The ratio of concentration between maternal muscle tissue and fetal trunk tissue varies from 10:1 to 20:1.

7. The pattern of distribution of the gonadotropin suggests that the chorion releases the hormone into the maternal blood. Secondarily, some of it passes the "placental barrier" and enters the fetal system across the wall of the chorionic vesicle.

8. The concentrations of gonadotropin slope similarly in all fetal and maternal tissues and fluids from the eleventh week until parturition, falling to about one twenty-fifth of their original values.

9. After therapeutic abortion the maternal system is cleared of the stored chorionic gonadotropin within the same length of time as after normal delivery.

10. The hypophysis of the pregnant woman seems to discontinue production of gonadotropins completely. If, however, a pregnant uterus is removed, the hypophysis resumes its normal functions within not more than six days.

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PROLONGED TREATMENT OF A CASE OF PERIARTERITIS NODOSA WITH ACTH: THE EFFECTIVE DOSE AS MEASURED BY METABOLIC BALANCES*†

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THIS report describes the metabolic and clinical effects of prolonged treatment with ACTH[1] (adrenocorticotropic hormone) in a patient with periarteritis nodosa. The decision to administer the hormone was based on the premise that the disease is one primarily involving the collagen system (1). Other diseases (2–8) characterized by changes in the collagen or ground substances have been found to respond favorably to ACTH or cortisone but at the time this study was undertaken there had been no published work on the effect of either of these agents in periarteritis nodosa. Although the metabolic responses to both hormones are now well described, the investigation was planned to include a fairly complete survey of the clinical and laboratory aspects of treatment in the hope that some additional information on the mechanism of the therapeutic action of adrenocorticotropin would be obtained.

CASE REPORT, W.K.A.

A 58-year old white male accountant entered Wadsworth Hospital on April 27, 1949, with a four-year history of increasingly severe asthma. Paresthesia of the feet developed six months prior to admission, with progressive weakness and right foot drop. Both hands became similarly involved one month before hospitalization. He lost his appetite and had a low grade fever. Because of food faddism, his diet had been poor for some time.

Received for publication September 12, 1950.

Reviewed by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions of the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

* This study was supported in part by a grant from the American Cancer Society on Recommendation of the Committee on Growth of the National Research Council.

† A preliminary report of this work has appeared in Proceedings of the First Clinical ACTH Conference, The Blakiston Co., Philadelphia, 1950.

[1] The ACTH used in this study was supplied by the Armour Laboratories, Chicago, through the courtesy of Dr. John R. Mote.

Physical examination on entry revealed a well-developed male, measuring 180.8 centimeters in height, weighing 50 kilograms, who was malnourished to the point of cachexia (Fig. 1). His temperature was 99° F., pulse 90, respirations 24 and blood pressure 130/70. The tongue was thick, smooth, and beefy red, with fibrillary twitching. The pharynx was red, smooth and moderately injected. Nasal polyps and edema of the nasal mucosa were present. The chest was sunken and wasted. Numerous scattered musical rales and rhonchi were present. The area of cardiac dullness appeared slightly decreased but no other abnormalities of the cardiovascular system were noted. No masses or other ab-

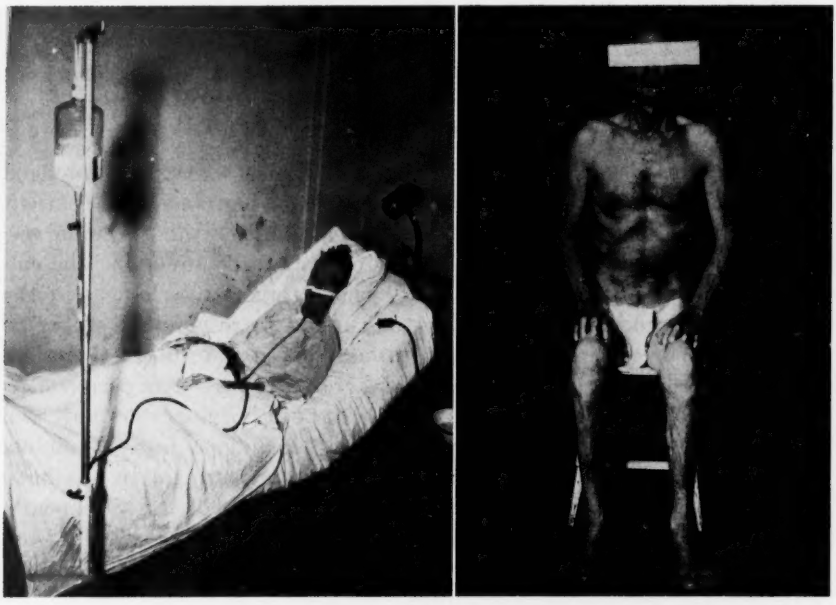

FIG. 1. *Appearance of patient.* Picture to left taken before ACTH therapy was begun. Picture on the right depicts status (July 1950) six months after completion of the study.

normalities of abdominal organs were palpable. Slight tenderness was noted on palpation of the right epididymis. Wasting was evident in all extremities. The skin over the distal phalanges was smooth and somewhat depigmented. Both wrist joints and all of the interphalangeal joints were stiff. Patellar and Achilles reflexes were absent bilaterally, but no pathologic reflexes were demonstrable. Right foot drop was present and bilateral quadricep weakness was marked. Vibratory sense was impaired over both feet and ankles, and pain sense was dulled in the same areas and over the ulnar distribution of both hands and wrists. A firm lymph node, 1 cm. in diameter, was palpable in the right axilla. The remainder of the examination revealed nothing abnormal.

Examination of blood: The red blood cell count was 4.7 million per cu. mm.; hemoglobin, 90 per cent; white blood cell count, 12,000 to 31,000 with 40 to 50 per cent eosinophils; and a typical differential count—neutrophils 41, eosinophils 46, lympho-

cytes 6, monocytes 6, and basophils 1 per cent, respectively. Platelets were adequate and the erythrocyte sedimentation rate was 21 mm. per hour (Wintrobe). Normal values were obtained for serum protein, chloride, CO_2 content, uric acid and creatinine. The results of serologic tests were negative. Spinal fluid was normal. Urinary 17-ketosteroid excretion ranged from 0.78 to 4.2 mg. per twenty-four hours. Upon clinical examination, urine and stools were normal. Gastric analysis showed free acid.

X-ray examination of the chest revealed advanced pulmonary emphysema with a small elongated cardiac silhouette. A series of roentgenograms of the skull showed

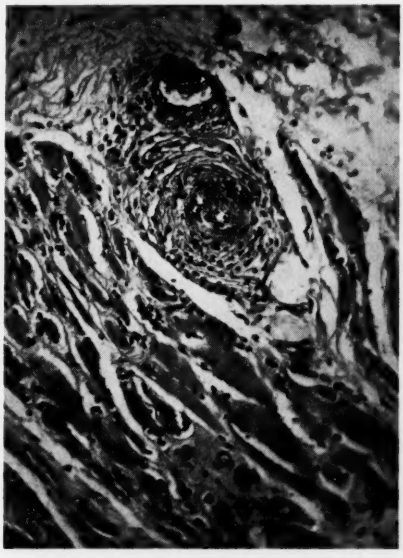

FIG. 2. *Biopsy specimen of gastrocnemius muscle before treatment.* The photomicrograph shows typical arteritis.

nothing abnormal. X-ray examination of the hands and feet showed generalized bone decalcification with two small cystic areas in the proximal phalanx of the left fourth digit.

Diffuse ST and T wave changes were present in the EKG, but no diagnostic pattern was apparent.

The nasal polyps were removed surgically and showed chronic inflammation with numerous eosinophils. A biopsy specimen from the gastrocnemius muscle gave the histologic picture characteristic of periarteritis nodosa (Fig. 2).

Treatment on the hospital ward was directed toward improving the patient's nutritional status with a high calory diet and vitamin supplements. The asthma was unsuccessfully treated with iodides, antispasmodics and antibiotics. Despite some initial weight gain, there was no sustained improvement. He remained chronically febrile. After four months of observation, the patient was transferred to the Metabolic Unit for further study and treatment.

PROCEDURE AND METHODS

A metabolic balance study was conducted for twenty-nine consecutive five-day periods, for a total of one hundred and forty-five days. During the first sixty-five days (periods 1 through 13), the diet consisted solely of a specially prepared liquid formula (Diet I). For the remaining eighty days (period 14 through 29), the formula was supplemented with certain foods (Diet II). The composition of the diet was determined by complete chemical analysis. Table 1 indicates the composition of Diets I and II.

TABLE 1. DIET

Composition diet of	Diet I	Diet II	During period 2
Protein	70.60	121.88	
Carbohydrate*	177.00	239.00	
Fat*	133.00	180.00	
Calories	2200.00	3100.00	
Na	1.077	2.421	0.350
K	2.484	3.880	0.858
Ca	1.712	1.934	0.604
P	1.795	2.528	0.630
N	11.30	19.50	3.860

* Estimated from standard dietary tables.

During the time that the patient received Diet I (period 1 through 13), ACTH[2] was given in two fifteen-day courses (periods 5–7 and periods 10–12 inclusive) in doses of 40 mg. per day (10 mg. every six hours) intramuscularly. After institution of Diet II (periods 14 through 21), the patient was given no ACTH for the second control period (periods 14 through 16). Then ACTH was given continuously for fifty days (period 17 through 26). The doses were increased and decreased every ten days in stepwise fashion as follows: periods 17 and 18—10 mg. per day; periods 19 and 20—20 mg. per day; periods 21 and 22—40 mg. per day; periods 23 and 24—20 mg. per day; and periods 25 and 26—10 mg. per day.

During periods when no ACTH was administered the patient received 0.2 unit of pitressin every six hours, an amount equivalent to the posterior pituitary hormone contaminating the ACTH used. The patient was not informed whether he was receiving pitressin or ACTH.

The following analyses were made: The urine was analyzed daily for

[2] The dosage of ACTH as referred to in this report is expressed in terms of the Armour La-1A standard.

uric acid, N, P, Na, K, Cl, glucose, creatine and creatinine. Daily circulating eosinophil counts were performed. Determinations of blood uric acid, glucose, NPN, glutathione, Na, Cl, K, Ca, P, CO_2, creatinine, proteins, blood count and packed cell volume were made every five days. Stool and urine collections, pooled by the period, were analyzed for N, Ca, P, Na, Cl, and K. The "11-oxysteroids" and 17-ketosteroids were determined in the 24-hour urine at least once in each period. Glucose tolerance tests, sternal marrow aspiration and study of sweat electrolytes were carried out at appropriate intervals.

Analytic methods were as follows: Aliquots of diet, feces, urine, emesis and sputum were ashed in a muffle furnace at a temperature not exceeding 450° C. Sodium and potassium determinations were performed directly on diluted specimens of urine, using the Beckman flame photometer; in the case of other minerals, the elements were determined on solutions of ash. The method of Washburn and Shear (9) was used for the determination of calcium, and phosphorus was ascertained both by the gravimetric method of Washburn and Shear and the colorimetric method of Fiske and SubbaRow (10). Nitrogen was determined by the Hiller, Plazin and Van Slyke modification of the Kjeldahl procedure (11). Chlorides were determined by modifications of the Volhard (12, 13, 14) silver nitrate-ammonium thiocyanate titration; protein by the method of Howe (15); NPN by the method of Koch and McMeekin (16); creatinine by the method of Bonsnes and Taussky (17); 17-ketosteroids by the method of Pincus (18) and Drekter *et al.* (19); creatine by the method of Clark and Thompson (20); CO_2 by the method of Van Slyke (21); blood sugar by the method of Kingsley and Reinhold (22); serum calcium by the method of Van Slyke and Sendroy (23); "11-oxysteroids" by the method of Heard, Sobel and Venning (24); uric acid by the method of Folin (25); eosinophil count by the method of Dunger (26) with modifications suggested by Thorn *et al.* (27); glutathione by the method of Woodward and Fry (28); and sweat electrolytes by the method of Leslie and Levin (29).

RESULTS

Clinical

While receiving ACTH the patient noted a feeling of well-being with improvement in appetite and strength. Dyspnea became less, and although increased pain and paresthesia of the extremities were noted for the early period of therapy, these gradually improved. It is worthy of emphasis that only due to the early increase of appetite following ACTH could Diet II be administered.

Objectively, the severe asthma disappeared promptly, with reduction in sputum from 450 cc. daily to a trace, and clearing of bronchial rales.

A gradual increase in the ability to use the extremities in walking and typing were observed. There was little objective improvement in neurologic findings. As noted in Figure 3, the temperature and pulse dropped to normal ranges during ACTH therapy, while the blood pressure increased

slightly. There was a decided improvement in the patient's appearance (Fig. 4). The complexion became ruddy, the face was fuller, and the weight increased moderately (Fig. 5). Although the original muscle biopsy specimen showed typical arteritis, specimens obtained on three occasions subsequent to ACTH therapy gave negative findings.

Symptoms and signs suggesting temporary hypoadrenocorticalism were evident for twenty-four to forty-eight hours each time the hormone was withdrawn. The blood pressure fell to about 90/60 associated with weak-

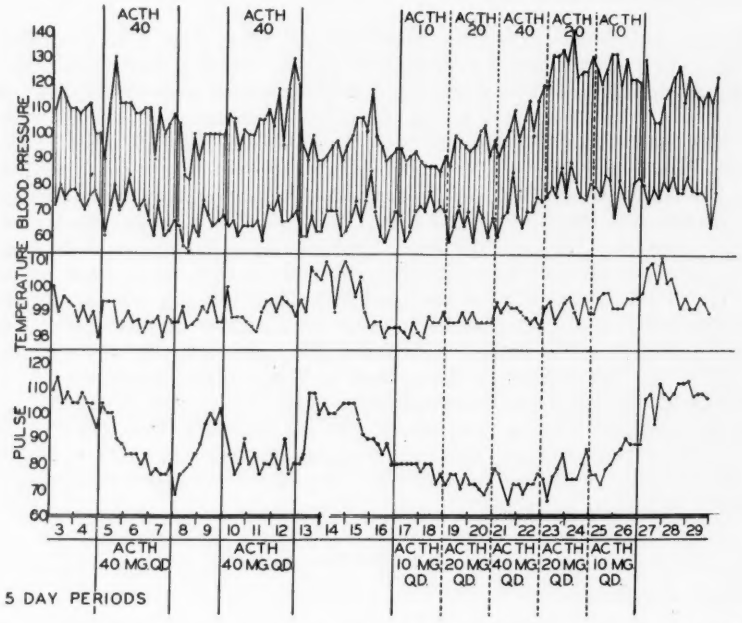
ACTH 40
ACTH 40
ACTH 10
ACTH 20
ACTH 40
ACTH 20
ACTH 10
BLOOD PRESSURE
TEMPERATURE
PULSE
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
ACTH 40 MG QD
ACTH 40 MG QD
ACTH 10 MG QD
ACTH 20 MG QD
ACTH 40 MG QD
ACTH 20 MG QD
ACTH 10 MG QD
5 DAY PERIODS

FIG. 3. *Blood pressure, temperature and pulse record.* Note: a) Tendency for blood pressure to increase on prolonged administration of ACTH (periods 17–26) and temporary hypotension on withdrawal. b) Disappearance of low-grade fever during ACTH therapy and reappearance of fever on withdrawal. c) Decline in pulse rate during ACTH therapy, with tachycardia on discontinuance of hormone.

ness, lassitude and anorexia. As readjustment took place, these manifestations gradually improved. A thrombophlebitis of the right leg developed during ACTH administration but cleared without complication.

About four weeks after discontinuing ACTH therapy, upon completion of the study, dyspnea, wheezing and sputum returned. A further course of the hormone in doses of 20 mg. per day for two weeks followed by 10 mg. per day for one week caused these symptoms to subside.

At the present time, fourteen months after completion of this study, the patient has maintained much of the described benefit (Fig. 1).

Hematologic

The total white blood cell count varied considerably before treatment, and it is impossible, therefore, to ascribe moderate changes to therapy.

FIG. 4. *Appearance of patient during therapy.* Picture on left depicts general improvement seven days after ACTH was begun; and picture on right shows the patient at completion of study.

However, while receiving ACTH, there was a slight but definite tendency toward leukocytosis with a relative lymphopenia.

The most striking hematologic changes occurred in the levels of circulating eosinophils. Figure 6 shows the relation between eosinophil count and ACTH therapy. The eosinophils decreased dramatically with ACTH and disappeared from the peripheral blood after forty-eight hours. They did not reappear until two days after discontinuing therapy. During the post-treatment control periods the initial very high eosinophil counts were not duplicated. On the increasing-dose schedule, the eosinophils de-

creased in a stepwise manner, and gradually reappeared again after the study was completed. Their number was directly correlated with the return of the patient's asthmatic signs and symptoms.

The bone marrow, which was heavily infiltrated with eosinophils in all stages of development, also showed a change with ACTH therapy, in that marrow eosinophilia was slowly reduced during a period of six days. This

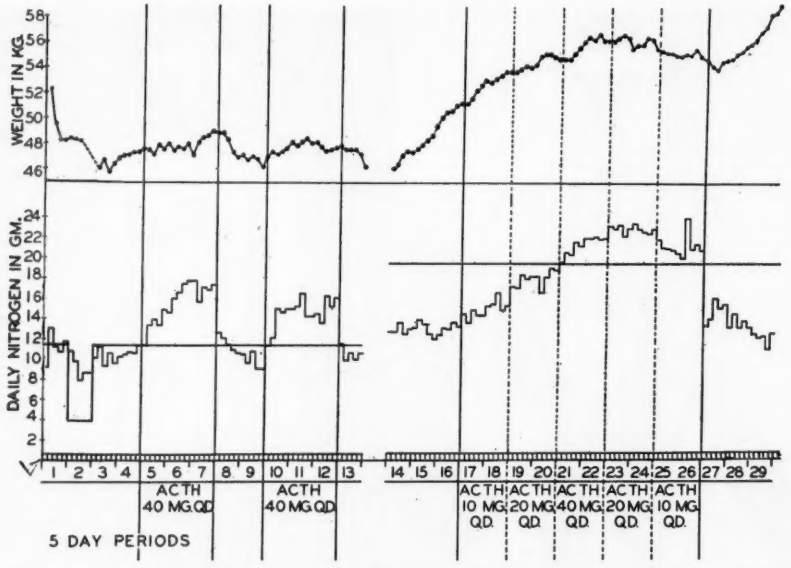

FIG. 5. *Daily nitrogen balance and weight curves.* The level of nitrogen intake is indicated by the straight line at 11.3 grams for periods 1 and 3–13 and by the similar line at 19.5 grams for periods 14–29. Fecal nitrogen is represented by the hatched area at the bottom of the graph, urinary nitrogen by the irregular line; the daily output is plotted so that there is a summation of urinary and fecal nitrogen. Extension of the urinary line above intake indicates negative nitrogen balance; where it falls below intake, the balance is positive.

Note: a) Essential nitrogen equilibrium in control periods 1 to 4, 8 to 9, and 13, except for period 2. Negative balance in this period due to decreased N intake associated with asthmatic attack; in periods 5 to 7 and 10 to 12, negative balance is due to administration of ACTH. b) With increased caloric and protein intake in periods 14–16, most of the supplementary nitrogen is stored, leading to considerable gain in weight. c) The effect of 10 mg. of ACTH is to increase urinary nitrogen and decrease nitrogen storage. In spite of the need for protein, larger doses of ACTH progressively increase the urinary excretion of nitrogen until the balance is again negative. d) Once the adrenal cortex has received sufficient stimulation, the 10 mg./day dose of ACTH produces negative nitrogen balance (periods 25 and 26). e) With withdrawal of ACTH, nitrogen excretion drops to pretreatment levels and weight gain begins again due to storage of protein.

indicates that the eosinopenic effect of ACTH may be based, at least in part, upon marrow inhibition.

Metabolic data

Weight and nitrogen balances

The weight chart (Fig. 5) reveals no significant gain in weight during the first sixty-five days, in spite of definite clinical improvement. The minor fluctuations that were observed were correlated with sodium and chloride

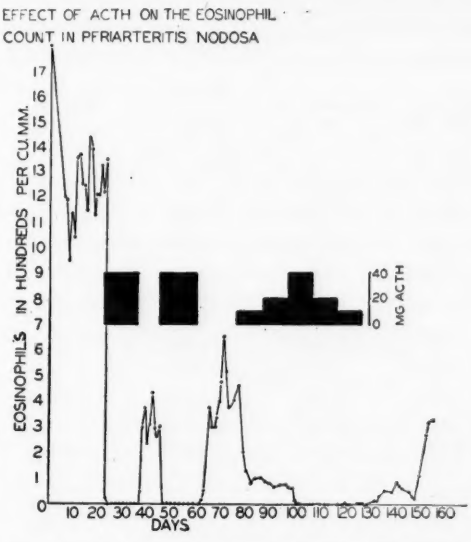
EFFECT OF ACTH ON THE EOSINOPHIL COUNT IN PFRIARTERITIS NODOSA
EOSINOPHILS IN HUNDREDS PER CU.MM.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
40
20
0
MG ACTH
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
DAYS

FIG. 6. *Eosinophil counts.* A dose of 40 mg. of ACTH daily was required to suppress the circulating eosinophils completely; but once suppressed, smaller doses maintained the effect.

retention and were probably due to temporary increase of fluid in the extracellular compartment.

The pronounced catabolic effect of ACTH upon nitrogen balance is also depicted in Figure 5. The negative nitrogen balance during treatment, without significant compensatory retention in control periods, is indicative of considerable destruction of protoplasm. In an already emaciated subject this may constitute an argument against prolonged continuation of this form of therapy in the presence of a restricted diet. The increased excretion of nitrogen was evident within the first forty-eight hours after treatment began and ceased abruptly when the hormone was withdrawn.

The beneficial effect of ACTH upon the patient's appetite made it pos-

sible for him to ingest Diet II. Associated with Diet II a strongly positive nitrogen balance occurred, with a weight gain of 5.2 kilograms in the first fifteen days (periods 14–16).

A comparison of the calculated gain in weight with that observed for the final phase of this study is given in Table 2. It is of interest that the

TABLE 2. TABULATION OF CALCULATED AND OBSERVED CHANGES IN BODY WEIGHT (FIVE-DAY PERIODS)

Period	Weight changes based on retention or loss of Cl and N				Medication
	Weight = Cl mEq. Cl/105	Weight = N Gm. N×27	Weight, total calculated	Weight, observed	
	grams	grams	grams	grams	
14	+1950	+858	+2708	+1556	
15	+1800	+872	+2672	+2440	
16	+ 276	+827	+1103	+1150	
					ACTH
17	+ 581	+705	+1286	+1750	10 mg./day
18	+ 143	+514	+ 657	+ 800	10 mg./day
19	+ 210	+270	+ 480	+1150	20 mg./day
20	− 543	+163	− 380	− 30	20 mg./day
21	+1228	−182	+1046	+1680	40 mg./day
22	+ 105	−192	− 87	− 220	40 mg./day
23	+ 371	−446	− 75	− 350	20 mg./day
24	− 57	−536	− 593	− 400	20 mg./day
25	− 47	−245	− 292	− 260	10 mg./day
26	− 380	−285	− 665	− 990	10 mg./day
27	+ 675	+642	+1317	+ 990	
28	+1714	+774	+2488	+1730	
29	+2220	+991	+3211	+3150	
Totals	*+10,246*	*+4730*	*+14,876*	*+14,146*	

marked and rapid gain in weight upon changing the diet was largely due to retention of extracellular water during period 14. Nitrogen retention also contributed to the weight gain but the effect appeared more slowly. The leveling off of the weight curve during prolonged ACTH administration is due to decreasing nitrogen retention. The reversal of the ACTH effect is seen in period 27, wherein a return to a sharply positive nitrogen

balance is observed. The over-all picture is one of a gain of 10 Kg. of extracellular fluid and 4.7 Kg. of protoplasm. The total of these gains in weight as calculated, pointed to an expected gain in weight of more than 14 Kg. The observed gain in weight was 14.1 Kg. This represents good correlation in view of the errors inherent in the assumptions upon which these calculations are based.

During the stepwise dose schedule of ACTH, the effect of even 10 mg. daily is reflected in a less positive nitrogen balance. With 40 mg. of ACTH per day, negative nitrogen balance occurred and persisted through decreasing doses. It would appear that once the adrenal cortex has been "primed" by full doses, smaller doses of ACTH are capable of exerting a continuing potent effect upon nitrogen exchange.

The data in period 2 were obtained while the patient was in status asthmaticus for these five days. Superficially the negative nitrogen balance appears to resemble the ACTH treatment periods; however, it is apparent that the negative nitrogen balance was not related to increased urinary loss but rather to decreased nitrogen intake. There was no change in eosinophil count accompanying this period of negative balance. The metabolic data do not suggest that the asthmatic episode was associated via stress with an increased production of endogenous ACTH.

Electrolyte changes

Sodium and chloride: The effects on the balances of sodium and chloride were qualitatively similar, but of different magnitudes, the shifts in sodium exchange being approximately twice as great as those of chloride (Fig. 7). There was a temporary moderate retention of sodium and chloride during the first days of each course of ACTH therapy, followed by an escape from adrenal cortical control as evidenced by re-establishment of equilibrium.

The most pronounced change in these electrolytes appeared within forty-eight hours of cessation of ACTH therapy, when a sharp salt diuresis occurred. On one occasion this diuresis exceeded the retention of the three previous periods of treatment. The total retention amounted to 343 mEq. of sodium and 148 mEq. of chloride, and the loss in about seventy-two hours was 418 mEq. of sodium and 187 mEq. of chloride. Although the over-all balance for fifty-five days (periods 3 through 13), during thirty of which ACTH was administered, showed a net retention of 228 mEq. of sodium and 63 mEq. of chloride, the positive balance may have been more apparent than real, since no accounting can be given of losses from the skin. It should be mentioned in this connection that the sweat electrolytes were strikingly affected during administration of ACTH and decreased in concentration from 30.8 and 24.0 to 6.2 and 2.6 mEq. of sodium chloride respectively, per liter.

When ACTH was given over a more prolonged interval, the patient was in an active phase of repletion (periods 17 through 26) and conspicuous retention of salt occurred only at the outset of treatment at a dose level of 10 mg. daily and again when the dose was increased to 40 mg. a day (period

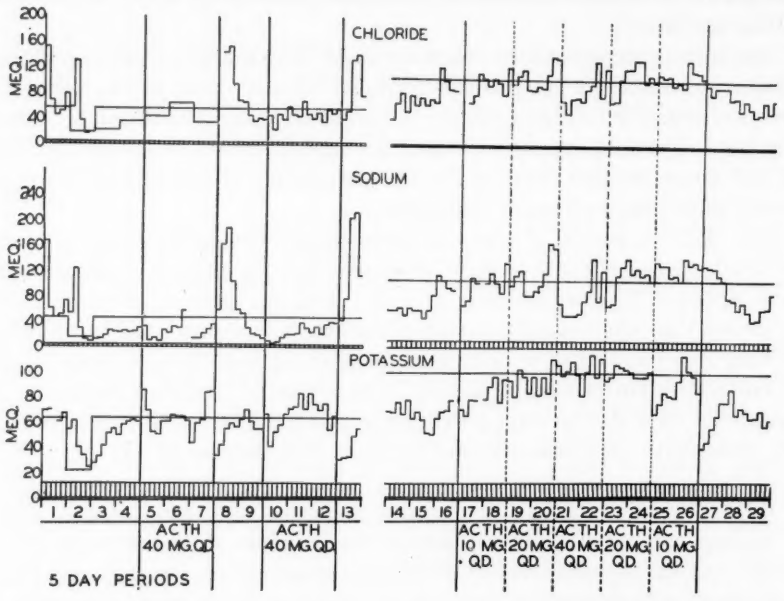

FIG. 7. *Electrolyte balances.* Five-day periods are numbered consecutively along abscissa. Manner of charting is similar to that used in Figure 5. Units of the scale are in milliequivalents.

Note: a) Sodium and chloride retention is indicated only at the higher level of dosage of ACTH and during periods of rapid repletion. Withdrawal of the 40 mg. dose of ACTH, with the patient in caloric and nitrogen equilibrium (periods 3–13), is followed by sodium and chloride diuresis. Gradual reduction of ACTH dosage, however, during the repletion phase, is not followed by salt diuresis. b) Potassium balance generally parallels nitrogen balance, except in periods 25 and 26, when potassium is retained subsequent to the reduction of ACTH dosage from 20 to 10 mg. daily. c) Negative balances of the three elements appear in period 2, and are associated with severe asthma and a lower dietary intake.

21). Reducing the dose to 10 mg. in periods 25 and 26 was followed by moderate salt diuresis. Stopping the hormone in period 27 was not associated with the usual large loss of electrolytes. The small doses of ACTH apparently failed to maintain the full activity of the adrenal cortex with respect to salt active corticoids, but did prevent the rapid regression in function that resulted from discontinuing the 40 mg. dose abruptly.

Potassium: The effect of ACTH upon potassium was in most respects opposite to that upon sodium and chloride (Fig. 7). An early potassium

diuresis occurred while salt was being retained, followed by potassium retention and then a gradual increase in urinary excretion until the balance became distinctly negative. The secondary loss of potassium seemed to be associated with the protein catabolic response, whereas the initial diuresis may have represented potassium derived from the extracellular fluid (30). Bartter *et al.* (31) have suggested that the phase of potassium

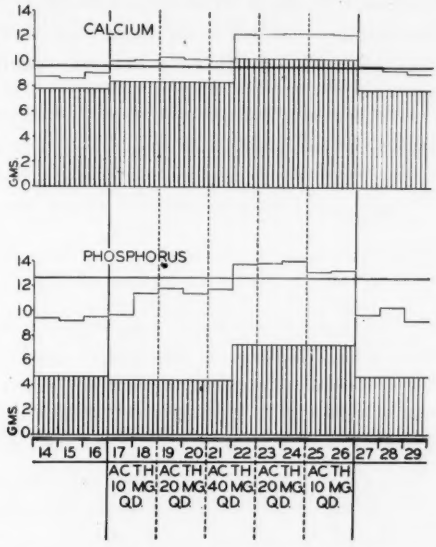
CALCIUM
PHOSPHORUS
GMS
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
ACTH 10 MG QD
ACTH 20 MG QD
ACTH 40 MG QD
ACTH 20 MG QD
ACTH 10 MG QD

FIG. 8. *Calcium and phosphorus balance.* Method of charting is similar to that used in Figure 5, except that all data are presented in terms of 5-day periods.

Note: a) Increased urinary excretion of both calcium and phosphorus during administration of ACTH. b) Prolonged administration of the hormone appears to increase the fecal excretion of both calcium and phosphorus. c) The over-all effect of ACTH is to produce negative calcium and phosphorus balances.

retention that takes place after the first twenty-four hours of therapy may result from the deposition of the element with glycogen (32). While on Diet I, essentially a maintenance diet, this effect was apparent during both courses of ACTH (periods 5 to 7 and 10 to 12, Fig. 7). The abrupt retention of potassium following cessation of therapy was striking and preceded diuresis of sodium chloride by about twenty-four hours. Both the clinical symptoms and these changes in urinary electrolytes are compatible with temporary suppression of adrenal cortical function.

The results of the more prolonged use of ACTH while the patient ingested Diet II are difficult to analyze. Rapid repletion and gain in weight due to water, electrolyte and nitrogen retention were under way prior to hormone administration. Moreover, the schedule of dosage as previously

mentioned was quite different. Hormonal withdrawal effects with potassium retention occurred in periods 23 and 25 (Fig. 7); otherwise the excretion in the urine followed the pattern of the nitrogen balance rather closely. The concentration of serum potassium decreased slightly under therapy but there was no true hypopotassemia.

Calcium and phosphorus balance: The data on calcium and phosphorus are given in Figure 8 and Table 3. It is apparent that ACTH produced a definite increase in urinary excretion of both calcium and phosphorus, expecially the latter. The calcium balance became positive only after the diet was increased.

TABLE 3. CALCIUM BALANCE DATA (PERIODS 1–13)

Period	Urinary Ca	Total Ca output	Ca balance
	grams	grams	
1	1.531	10.288	−1.728
2	1.244	5.284	−2.264
3	0.883	9.640	−1.080
4	1.395	10.302	−1.742
5	1.802	10.559	−1.999 ACTH
6	1.980	10.767	−2.207 ACTH
7	1.490	10.247	−1.687 ACTH
8	0.880	9.637	−1.077
9	0.742	9.499	−0.939
10	0.789	9.546	−0.986 ACTH
11	0.949	9.706	−1.146 ACTH
12	0.975	9.732	−1.172 ACTH
13	0.678	9.435	−0.775

With prolonged ACTH administration both calcium and phosphorus were excreted in increasing amounts as the dose was increased. There appeared to be a significant change in fecal excretion of calcium and phosphorus during periods 17 to 26. The average fecal loss of calcium and phosphorus per period in six control periods (14 to 16 and 27 to 29) was 7.847 and 4.734 grams respectively. In the first five treatment periods (17 through 21), the loss increased to 8.415 Gm. for calcium while decreasing slightly for phosphorus to 4.406 Gm. During the final twenty-five days the period losses were 10.261 Gm. for calcium and 7.267 Gm. for phosphorus.

Both the calcium and phosphorus content of the serum seemed to be affected by ACTH. Initial base line values of 10 and 4 mg. per cent respectively fell to an average of about 9 and 3 mg. per cent. With the diet change, these values rose to their pretreatment range. Continued therapy

again produced a progressive decline to 8.4 and 2.5 mg. per cent for serum calcium and phosphorus respectively. During the final two periods of 10 mg. of ACTH daily, the serum calcium rose to 9 mg. per cent while the phosphorus remained at the low level of 2.5 mg. per cent. Upon withdrawal of the hormone, the serum calcium remained at 8.6 mg. per cent while the blood phosphorus rose to 3.9 mg. per cent. The mechanism responsible for these changes is not clear.

In summary, ACTH produced a definite increase in fecal and urinary loss of calcium and phosphorus. It is also noteworthy that for these minerals, as well as for nitrogen, once full effect was obtained by 40 mg. of ACTH per day, successive decrements in dosage were not accompanied by proportionate decreases in the excretion. Therefore, the effect of ACTH upon calcium and phosphorus excretion is apparently maintained for some time with small doses, once the adrenal cortex has received sufficient stimulation.

Uric acid, creatine and creatinine

The effects of ACTH upon uric acid, creatine and creatinine are charted in Figure 9. The blood creatinine did not vary significantly during the study, and except for a gradual slow rise with prolonged treatment, ACTH had no effect upon urinary creatinine.

Urinary creatine and uric acid paralleled one another. ACTH gave rise to a significant increase in excretion of each. This effect was stepwise in intensity during the increasing dosage schedule, was maximal with 40 mg.

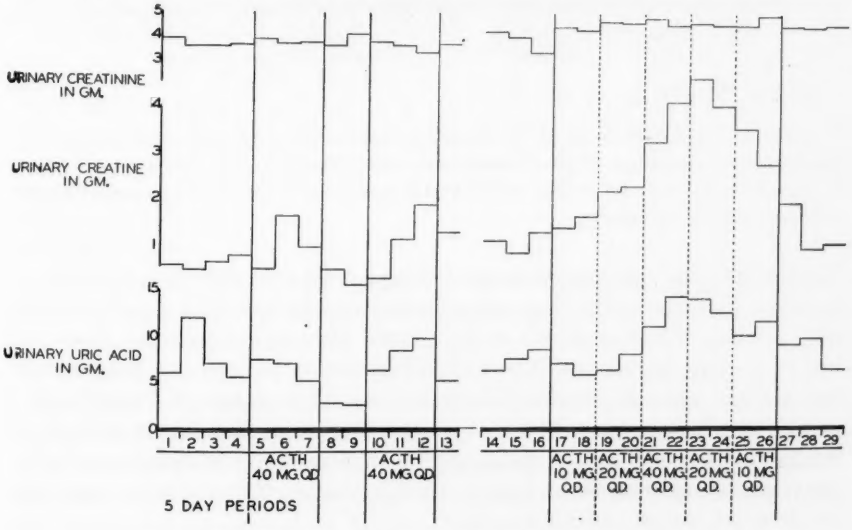
URINARY CREATININE IN GM.
URINARY CREATINE IN GM.
URINARY URIC ACID IN GM.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
ACTH 40 MG QD
ACTH 40 MG QD
ACTH 10 MG QD
ACTH 20 MG QD
ACTH 40 MG QD
ACTH 20 MG QD
ACTH 10 MG QD
5 DAY PERIODS

FIG. 9. *Excretion of creatinine, creatine and uric acid in urine.*

per day, and upon reduction of ACTH the maximal effect was practically fully sustained. The urine and serum uric acid changes were similar to that noted by others (33).

"11-Oxysteroids" and 17-ketosteroids

Figure 10 depicts the effect of ACTH upon urinary 17-ketosteroids and "11-oxysteroid" excretion. The low basal level of 17-ketosteroid excretion in this patient was no doubt associated with general malnutrition. Although complete data on 17-ketosteroids are not available, scrutiny of the

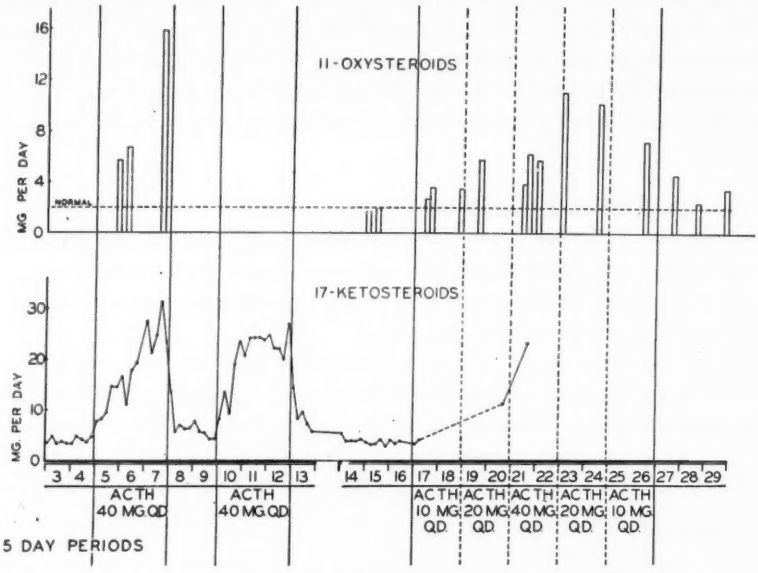

FIG. 10. *Urinary excretion of 11-oxy and 17-ketosteroids.* The plotted data represent total 24-hour excretions of the respective steroids. The excretion of both 11-oxy and 17-ketosteroids increased rapidly with ACTH and returned to the base line after the hormone was discontinued.

"11-oxysteroids" during prolonged therapy with ACTH demonstrates a stepwise increase as the dose was progressively raised, with a peak of 11.6 mg. per day. As is apparent in the graph, although decreasing doses did result in some decrease in "11-oxysteroid" output, comparable doses on the descending schedule resulted in about twofold increases of "oxysteroid" excretion when compared to that on the ascending dose schedule. This is further evidence that once the proper mechanisms are activated by ACTH, they can be maintained at nearly full functional efficiency with comparatively smaller doses of the hormone.

Glucose and glutathione studies

Hyperglycemia and glycosuria developed each time ACTH was administered. Figure 11 illustrates the diabetogenic effect of ACTH upon carbohydrate metabolism.

The blood glutathione (actually the method used titrates sulfhydryl radicals, which presumably indicate the level of glutathione) decreased with the first course of ACTH from 61 and 71 mg., to 49 mg. per cent. Subsequent administration of ACTH, either for short-term or prolonged courses, produced no significant alteration in glutathione. Diuresis of uric

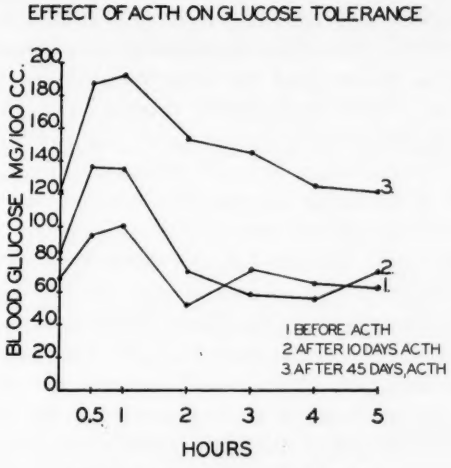

FIG. 11. *Glucose tolerance curves.*

acid and the diabetogenic effect of ACTH were demonstrable each time it was given. This disparity between the level of glutathione, uric acid excretion and glucose tolerance does not agree with Conn's (34) hypothesis concerning the diabetogenic effect of ACTH.

Blood changes

No significant alterations in packed cell volume, plasma protein, CO_2, or NPN were noted throughout the course of study.

DISCUSSION

ACTH, in the doses given, stimulated the adrenal cortex to elaborate three principal types of steroid hormones as measured by the metabolic and hematologic changes. Although this brought about an amelioration of the disease, no evidence was uncovered pointing to a dysfunction of the pituitary-adrenal axis which might be considered etiologically significant

in pathogenesis. Certainly our patient demonstrated the same effects from ACTH that have been reported in normal individuals by others (30, 33, 35). As in other collagenous diseases, the beneficial effects were mediated through as yet unknown mechanisms. It is also true that when ACTH was discontinued the signs and symptoms slowly recurred. Although biopsy evidence of the disorder seemingly disappeared, longer follow-up and more cases will be necessary before final appraisal of the value of ACTH can be definitely established. The sensitive response of the circulating eosinophils to ACTH stimulation as observed in this patient, recommends the use of the eosinophil count in assessing the degree of adrenal cortical activity in this case. Other experience with various patients studied in this laboratory does not demonstrate this close correlation of eosinophils to adrenal cortical response, as determined by other parameters. The clinical and metabolic effects of ACTH were closely related to the dosage used. It is unfortunate that larger doses were not given, to learn if 40 mg. per day produced maximal stimulation. It is of interest, however, that the clinical benefits and most of the metabolic changes induced by the 40 mg. dosage were essentially maintained on successive decrements to 20 and 10 mg. per day. The chief exception appeared in the electrolyte exchanges. The balances of sodium, chloride and potassium were of the post-treatment type on the decreasing schedule of dosage. Since edema is one of the undesirable side actions of treatment, escape from excessive DOCA effect may be advantageous. From the observations cited, maintenance of adequately high levels of adrenal cortical function with relatively smaller amounts of ACTH seems feasible after the gland has been stimulated with sufficiently large priming doses. The therapeutic implication of this concept is obvious.

SUMMARY AND CONCLUSIONS

1. Metabolic studies of the effect of ACTH in a patient with periarteritis nodosa have been continued for one hundred and forty-five days.

2. Clinical and pathologic remission occurred; cure did not take place but marked physical improvement has been sustained for fourteen months.

3. The metabolic responses to the hormone were similar to those observed in normal individuals.

4. Changes in the eosinophil count were correlated closely with metabolic effects and clinical progress.

5. After adequate "priming" of the adrenal, decreasing doses of ACTH were not accompanied by proportional decreases in the signs of metabolic activity.

6. Administration of 40 mg. of ACTH daily produced a strongly negative nitrogen balance while the patient was ingesting a maintenance diet; this dosage was also sufficient to negate completely the marked nitrogen reten-

tion induced by a high protein, high caloric diet. Evidence is presented that this effect was due to the hormone and not merely to repletion of body protein.

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THE RESPONSE OF PATIENTS WITH BRONCHIAL ASTHMA TO EPINEPHRINE AND TO ADRENOCORTICOTROPIC HORMONE

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DURING the past decade several authors have suspected that endocrine regulations participate in the allergic syndrome. Rackemann (1) in particular, emphasized the importance of nonspecific factors in the etiology of bronchial asthma and presented evidence for adrenal cortical deficiencies in 1 isolated case. He suggested that bronchial asthma might be one of the "diseases of adaptation" characterized by Selye (2).

In order to evaluate further the significance of their findings, clinical, metabolic and hematologic changes were followed in patients with bronchial asthma before, during, and after therapy with epinephrine, adrenocorticotropic hormone (ACTH), and ACTH combined with ascorbic acid. Epinephrine and ACTH were employed in order to study the effect of compounds acting at various levels of the pituitary-adrenal axis. Ascorbic acid was given for two reasons: first, to insure that, in all stages of the experiment, sufficient Vitamin C would be available to allow for synthesis of adrenal cortical hormone; and second, to study the effect of large doses of Vitamin C on adrenal cortical mechanisms in man, since Dugal and Thérien (3) had observed that ascorbic acid prevented the typical enlargement of the adrenal in animals under stress.

Bordley and his co-workers (4), Randolph (5) and our own studies (6) have established, during the past year, that patients suffering from bronchial asthma respond favorably to the administration of ACTH. The available data make it evident that most patients thus investigated appear to have adrenal glands which respond to stimulation by the pituitary hormone. It seems important, therefore, to report this study on 2 patients who—treated at the same time, with identical amounts of identical lots of the purified extract available to us—showed marked differences in hematologic and hormonal changes following injection of epinephrine and ACTH.

Received for publication September 8, 1950.

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METHODS

Selection of patients

The 2 patients who were studied (T. S., a woman 47 years of age; and W. P., a man 58 years of age) were ill with severe perennial bronchial asthma of allergic origin.

Each of the patients had suffered from the disease for at least ten years. They had been under observation in the Allergy Clinic, Research and Educational Hospitals, University of Illinois, for more than three years. They had comparable impairment of the respiratory function. They gave positive skin reactions to various inhalant allergens, including atmospheric molds prevalent in the Midwest (hormodendrum, aspergillus, alternaria and pencillium). They had been treated with increasing amounts of the allergens for a considerable period without satisfactory results. A brief history of each patient is appended at the end of this paper.

Clinical studies

The patients were studied simultaneously in the Metabolic Ward of the Research and Educational Hospitals, of the University of Illinois. They lived in airconditioned rooms, kept at a constant temperature of +74°F. Their basic regimen consisted of complete bed rest save for going to the toilet, and a fixed diet which provided each day: basal plus 40 per cent of requirement for calories; protein, 1 gram per kilo of body weight; 30 per cent of calories as fat and the remainder as carbohydrates. The vitamin C content of this diet was calculated from diet tables (7) to contain approximately 30 mg. of ascorbic acid per day. There was little food waste. Water intake was not fixed. Neither drugs, nor dietary supplements were administered save as noted in the text.

The patients were examined twice daily, in the morning and in the late afternoon. Subjective and objective changes were carefully recorded. Routine physical survey was completed during the first three days after admission, followed by continuous studies whenever necessary.

Determinations of vital capacity and breathing reserve (8, 9) were carried out on a Leitz-Bergmann spirometer and on a Collins respirometer with a capacity of 9 liters, using a two-speed kymograph. Breathing reserve (B. R.) is a function of minute volume (M. V., in liters of air) and maximum voluntary ventilating capacity (M. V. V. C., in liters of air). It is calculated from the formula:[1]

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[1] Although it is believed that the method has only limited value for making a comparison of respiratory measurements in different individuals, it seems adequate in the hands of skilled personnel, for recording progressive respiratory changes in a single individual.

$$\text{B.R.} = \frac{\text{M. V. V. C.} - \text{M. V.}}{\text{M. V. V. C.}} \times 100.$$

Biochemistry

The 24-hour urinary excretion of 17-ketosteroids was determined by the method of Cahen and Salter (10). Urinary excretion of 11-oxycorticosteroids and "11-desoxycorticosteroids" was measured by a modification of the method of Daughaday and his co-workers (11). This method measures the amount of formaldehyde liberated by the oxidation of urinary extracts with periodic acid. The 11-oxycorticosteriods are present in the water phase and "11-desoxycorticosteroids" in the benzene phase of the extracts. It is believed that the benzene phase contains steroids other than 11-desoxy compounds. For this reason, the chemical name "11-desoxycorticosteroids" has been placed in quotation marks throughout this paper. The fasting blood glucose (12) and free and esterified cholesterol (13, 14) were measured by standard techniques. Determinations of serum gamma globulin were made on blood taken at 7:30 A.M. and four hours after medication.[2]

Hematologic studies

Blood samples for absolute counts of circulating eosinophils were obtained at 7:30 A.M. and four hours after medication.

During the initial phase of the study the absolute eosinophil counts were made with Dunger's original method (16). In Dunger's technique, cells are lysed and clusters of eosinophilic granules are counted. Rapid clumping and evaporation make it necessary to complete the counts within a few minutes in order to get accurate and reproducible figures. Randolph's (17) technique avoids these disadvantages and was used in place of Dunger's method in the latter half of the study.

Studies were carried out on one marrow obtained by aspiration prior to, and at various intervals after, the administration of adrenocorticotropic hormone. The evaluation of the number of eosinophils in the bone marrow was made on stained smears, counting 2,000 cells (Best and Samter (18)).

The following preparations were used for therapy: adrenocorticotropic hormone (ACTH),[3] 50 mg.; ACTH, 50 mg. in combination with ascorbic

[2] We are very much indebted to Drs. H. Popper and G. de la Huerga who carried out the determination of serum gamma globulin with their turbidimetric method (15).

[3] Adrenocorticotropic hormone derived from equine hypophyses was kindly supplied by Dr. Irwin C. Winters of G. D. Searle and Company. They reported that "seven gamma" of the preparation caused a 25 per cent drop in the adrenal ascorbic acid of hypophysectomized rats. In comparison, Armour's standard (LA-1) produced similar response in two-gamma quantities. Each milligram of the preparation contained 0.01 U.S.P. unit of oxytocin.

TABLE 1. URINARY VOLUME, HORMONE EXCRETION AND EOSINOPHIL COUNTS IN T. S., ILL WITH ASTHMA AND TREATED WITH ACTH, ASCORBIC ACID AND EPINEPHRINE

Day of study	Treatment	Urinary excretion of			Eosinophils per mm.³		Urine volume ml./24 hrs.
		17-keto-steroids, mg./24 hrs.	11-oxycorti-costeroids, mg./24 hrs.	"11-desoxy-cortico-steroids," mg./24 hrs.	7:30 A.M.	12:00 noon	
1	Control	5.9	0.59	0.12	—	—	1080
2	Control	6.72	0.51	0.19	231	220	1620
3	Control	6.03	0.62	0.14	—	—	1400
4	Control	5.91	0.53	0.17	—	—	2340
5	ACTH, 50 mg. plus vitamin C, 250 mg. 4 X daily	16.8	2.55	0.19	154	61	820
6					0	0	630
7		23.1	2.19	0.15	0	0	580
8		27.8	1.80	0.10	0	0	500
9	ACTH, 50 mg. X4 daily	24.7	3.61	—	6	0	1040
10	ACTH, 50 mg. X4 daily	28.1	—	—	0	6	1200
11	ACTH, 50 mg. X4 daily	30.2	3.91	0.26	0	0	1200
12	ACTH, 50 mg. X4 daily		3.90	0.24	6	6	1100
13	Control	6.8	0.69	0.20	6	11	1350
14	Control				231	214	2600
15	Control	6.1	0.74	0.17	203	165	2580
16	Control		0.61		148	115	2500
17	Control	7.2	0.69	0.22	—	—	2400
18	Control				—	—	2100
19	Control	7.0	0.51	0.18	105	93	2150
20	Control				198	84	1380
21	Epinephrine, 0.8 mg. X4 daily	7.9	0.52	0.24	171	66	2100
22	Epinephrine, 0.8 mg. X4 daily		0.74	0.29	143	66	1720
23	Epinephrine, 0.8 mg. X4 daily	8.4	0.88	0.32	170	88	1400
24	Epinephrine, 0.8 mg. X4 daily	11.3	0.91	0.18	—	—	2400
25	Control	9.2	0.54	0.14	143	149	1800
26	Control						1900
27	Control	7.4	0.48	0.18	187	170	1700
28	Control	6.8	0.53	0.53	198	—	2010

acid, 250 mg.; epinephrine hydrochloride, 0.8 mg. in a 1:1,000 aqueous solution. Medication was given intramuscularly at 8:00 A.M., 12:00 noon, 4:00 P.M. and 8:00 P,M. The individual schedule of treatment for each patient is shown on Tables 1 and 2.

RESULTS

Data from the 2 patients, T. S. and W. P., are summarized in Tables 1 and 2, which show days and schedule of treatment, 24-hour excretion of 17-

TABLE 2. URINARY VOLUME, HORMONE EXCRETION AND EOSINOPHIL COUNTS IN W. P., ILL WITH ASTHMA AND TREATED WITH ACTH, EPINEPHRINE AND ASCORBIC ACID

Day of study	Treatment	Urinary excretion of			Eosinophils per mm.³		Urine volume ml./24 hrs.
		17-ketosteroids, mg./24 hrs.	11-oxycorticosteroids, mg./24 hrs.	"11-desoxycorticosteroids," mg./24 hrs.	7:30 A.M.	12:00 noon	
1	Control	8.1	0.68	0.19	—	—	1840
2	Control	8.9	0.78	0.24	292	242	1300
3	Control	7.8	0.76	0.24	—	—	1170
4	Control	8.91	0.72	0.25	—	—	1460
5	Epinephrine, 0.8 mg. X4 daily	8.10	0.93	0.19	385	203	1290
6	Epinephrine, 0.8 mg. X4 daily	9.12	0.87	0.29	182	148	1500
7	Epinephrine, 0.8 mg. X4 daily	10.08	0.39	0.35	286	170	1215
8	Epinephrine, 0.8 mg. X4 daily				357	231	1480
9	Control	11.4	1.01	0.26	418	387	2200
10	Control	9.2	0.98	0.17	—	—	1390
11	Control				—	—	1220
12	Control	8.9	0.90	0.21	308	418	1815
13	ACTH, 50 mg. X4 daily	14.3	1.30	0.15	662	231	720
14	ACTH, 50 mg. X4 daily				346	55	500
15	ACTH, 50 mg. X4 daily	14.2	1.32	0.14	258	220	850
16	ACTH, 50 mg. X4 daily	11.1	1.28	0.16	302	176	1600
17	ACTH, 50 mg. plus vitamin C, 250 mg. X4 daily	12.3	1.12	0.21	234	214	640
18	ACTH, 50 mg. plus vitamin C, 250 mg. X4 daily	10.8	1.01		310	222	1600
19	ACTH, 50 mg. plus vitamin C, 250 mg. X4 daily	11.6	0.88	0.17	242	209	950
20	ACTH, 50 mg. plus vitamin C, 250 mg. X4 daily				314	218	3100
21	Control	8.0	0.71	0.23	423	408	2300
22	Control	9.4	0.65	0.19	440	418	1400
23	Control	8.9	0.82	0.20	495	—	1390
24	Control				—	—	1900
25	Control	7.8	0.77	0.16	—	—	1770
26	Control	8.5	0.70	0.18	—	—	1810
27	Control	—	—	—	—	—	1930
28	Control	—	—	—	—	—	1480

ketosteroids, 11-oxycorticosteroids, "11-desoxycorticosteroids," absolute eosinophil counts at 7:30 A.M. and 12:00 noon and the volumes of urine during the period of observation.

(*A*) *Patient T. S.*

After a control period of four days, Patient T. S. received ACTH in combination with ascorbic acid for four days and immediately thereafter ACTH without ascorbic acid for another four days. After a post-therapy

period of eight days, epinephrine hydrochloride was administered for four days. This was followed by a final period of four days when no drugs were given.

Clinical changes. Following the administration of ACTH, with or without ascorbic acid, the patient experienced an extraordinary feeling of well-being which was absent during the period of injection of epinephrine. Clinical improvement paralleled the recorded change in breathing reserve. Originally, the breathing reserve varied from 54 to 65 per cent of normal. Forty-eight hours after the initial injection of ACTH with ascorbic acid it rose to 74 per cent and remained between 74 and 82 per cent of normal while ACTH was given, as well as during the first five days of the post-therapy period, regardless of the hour at which the measurement was made. On the sixth post-therapy day it decreased to 68 per cent of normal and reached 64 per cent on the eighth day. Administration of epinephrine raised the breathing reserve to 72 per cent but this rise was temporary and contingent on the continuous injection of the drug. Respiratory function, studied in the morning prior to the first injection of epinephrine, showed a breathing reserve which varied from 52 to 66 per cent of normal. Biochemical observations are shown in Figure 1. It may be seen that the excretion of 17-ketosteroids, which initially was within normal range, showed a significant increase after the administration of ACTH alone. The changes in the excretion of 17-ketosteroids after the injection of epinephrine hydrochloride were not significant. The 11-oxycorticosteroids showed a moderate increase after the administration of ACTH plus ascorbic acid, followed by a marked increase after ACTH alone; thereafter, little or no change was noted. The "11-desoxycorticosteroids" did not change after administration of ACTH with ascorbic acid, but showed a moderate increase after the administration of ACTH alone, and after epinephrine hydrochloride. No significant changes were noted in blood glucose, in free and esterified cholesterol nor in gamma globulin levels during the day.

Hematologic observations. It is shown in Table 1 that twenty-four hours after administration of ACTH with vitamin C, the absolute eosinophil count in circulating blood fell to zero and remained at or near this level throughout the time during which ACTH with ascorbic acid, and ACTH alone, were given. On the morning of the second post-therapy day the level of circulating eosinophils reached 231 eosinophils per cu. mm. When epinephrine hydrochloride was injected, a drop in the number of circulating eosinophils occurred. This change was much less than that observed after injection of ACTH. The bone marrow smears failed to show any significant change in eosinophil count during the study.

(*B*) *Patient W. P.*

After an initial control period of four days, patient W. P. was treated with epinephrine hydrochloride for four days followed by a four-day post-therapy period. He then received injections of ACTH for eight days. During the first four days, the patient received ACTH without additional medication. Ascorbic acid was added during the second four days of ACTH administration, and was injected simultaneously with the hormone, but at different sites. A final period of eight days of observation completed the study.

The subjective and objective changes in the patient's clinical condition

were of a small degree. The feeling of well-being which was reported spontaneously by patient T. S. was absent in patient W. P. The following episodes of bronchial asthma occurred during the period of observation:

On admission, the breathing reserve of patient W. P. was 42 per cent of normal. Rest in the hospital for more than eight days prior to the study increased his breathing reserve to 62 per cent. Whenever injections of epinephrine hydrochloride were given, breathing

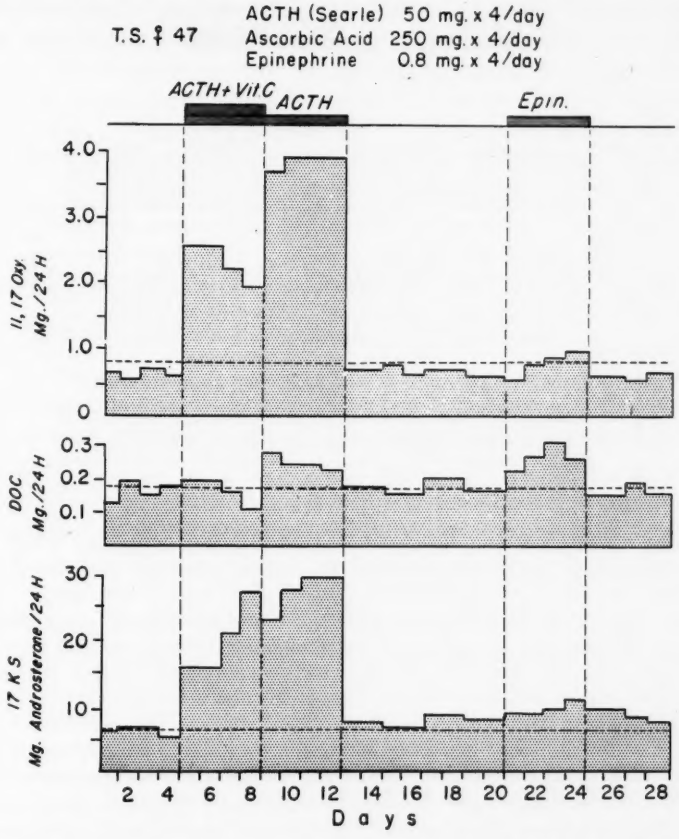

FIG. 1. Twenty-four-hour urinary hormonal excretion in Patient T. S., ill with asthma and treated with ACTH, epinephrine and ascorbic acid.

reserve increased from 66 to 78 per cent of normal. However, the change in breathing reserve lasted for only two hours and fell after this to pre-injection levels. On the fourth day of epinephrine administration, the patient had an attack of bronchial asthma which lasted for approximately three hours; 0.5 Gm. of aminophylline in 20 cc. of saline was then given intravenously and afforded relief within twenty minutes. Forty-eight hours after administration of ACTH alone, the breathing reserve increased to 70 per cent and

ranged from 64 to 74 per cent during the entire period of ACTH therapy. During the night following the first post-therapy day another severe attack of bronchial asthma developed, which lasted two hours and which responded promptly to intravenous administration of 0.5 Gm. of aminophylline in 20 cc. of saline. Breathing reserve during the

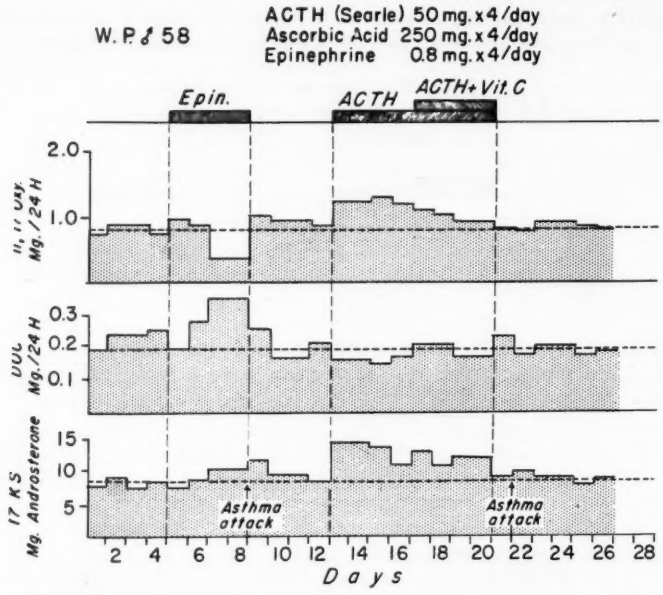

FIG. 2. Twenty-four-hour urinary hormonal excretion in Patient W. P., ill with asthma and treated with ACTH, epinephrine and ascorbic acid.

eight days after completion of therapy with ACTH and ascorbic acid ranged from 48 to 60 per cent. Biochemical changes are shown in Figure 2, which shows that the excretion of 17-ketosteroids rose slightly on the third and fourth day of injection of epinephrine hydrochloride and remained at this level through the first three days of the post-therapy period. Injections of ACTH produced a moderate increase in the excretions of 17-ketosteroids, but this decreased gradually during the continued administration of ACTH alone and ACTH plus ascorbic acid. The 11-oxycorticosteroid excretion was not affected by injection of epinephrine hydrochloride. There was a slight increase after the administration of ACTH alone, which was not sustained during continued administration of ACTH alone and ACTH with ascorbic acid. The "11-desoxycorticosteroids" increased moderately during the second, third, and fourth day of epinephrine injection, but during the administration of ACTH and ACTH plus ascorbic acid no changes in excretion levels were noted when compared with the control levels. No significant changes were seen in blood glucose, in free and esterified cholesterol nor in gamma globulin during the study.

Hematologic observations. The initial levels of circulating eosinophils found in patient W. P. were higher than those found in patient T. S. The fall of circulating eosinophils during injection of epinephrine hydrochloride was not very great. During the first two days of ACTH injections a moderate decrease in eosinophils was found, but during the

next two days and during the four days of administration of ACTH with ascorbic acid the eosinophils remained at control levels. Counts of bone marrow smears, obtained repeatedly throughout the period of study, failed to show any significant variation in the number or percentage of eosinophils.

DISCUSSION

Although 2 patients (T. S. and W. P.) suffering from chronic perennial bronchial asthma of allergic origin were observed under identical environmental conditions, the data obtained revealed marked differences in their responses to the administration of ACTH, of ACTH with ascorbic acid, and of epinephrine hydrochloride. In the first patient (T. S.) significant hormonal, hematologic and clinical changes were seen; but in the second patient (W. P.) these responses to therapy were weak. It must be assumed, therefore, that the amounts of hormone injected failed to stimulate W. P.'s adrenal cortex sufficiently, that his adrenal cortex was unable to supply the cortical steroids necessary to produce clinical and metabolic changes, or that the end organs were not responsive to the released hormone. The appearance of asthmatic attacks in this patient during continuous administration of epinephrine hydrochloride as well as after the administration of ACTH and ACTH with ascorbic acid, might be interpreted as the possible result of an exhaustion of adrenal cortical steroids, the release of which appears to have a profound effect on the clinical course of bronchial asthma in man.

The absence of adrenal cortical response in patients suffering from bronchial asthma is an unusual occurrence. It appears that most of the patients treated with ACTH have showed an adequate response as judged by depression of the number of circulating eosinophils, increase in the excretion of adrenocortical metabolites, and clinical improvement. Since glucorticoids are able to influence, favorably, the clinical picture of bronchial asthma, it seems conceivable that occasionally a patient might suffer from the disease because he is unable to supply an adequate amount of adrenal corticoids in spite of the ability of the anterior pituitary to release ACTH. The increase in 17-ketosteroids and 11-oxycorticosteroids following ACTH administration has been described by Forsham and co-workers (19) and by Mason and co-workers (20) and our data have agreed with these observations. On the other hand; in our study, ACTH failed to produce an increase in the excretion of "11-desoxycorticosteroids." The limitations in the method of determination of 11-oxycorticosteroids and "11-desoxycorticosteroids" make it difficult to interpret the significance of these results.

Therapy with ascorbic acid at the time of injection of ACTH apparently had little effect on the excretion of 17-ketosteroids, but a diminished excretion of 11-oxycorticosteroids was noted when this was done. These re-

sults are in accord with those obtained by Daughaday in scorbutic patients (21).

The effect of epinephrine therapy on the excretion of 17-ketosteroids, 11-oxycorticosteroids and "11-desoxycorticosteroids" in patient T. S. is noteworthy. Neither the excretion of 17-ketosteroids nor that of 11-oxycorticosteroids was significantly increased. The excretion of "11-desoxycorticosteroids," however, was increased. The significance of this is not understood.

The patients did not show the changes in blood sugar and esterified cholesterol reported by Conn (22) following the administration of ACTH. This is not surprising, since such changes seem to be highly individual responses to ACTH.

The hematologic change, *i.e.*, the decrease in circulating eosinophils, generally was proportional to the increase in the excretion of adrenal cortical metabolites. Bone marrow studies before and after administration of ACTH failed to show any significant alteration of the eosinophil count.

The clinical improvement and increase in breathing reserve in each patient seemed to depend on the presence of a satisfactory hormonal and hematologic response.

SUMMARY

1. The hematologic, hormonal and clinical changes following administration of ACTH, ACTH with ascorbic acid, and epinephrine hydrochloride are reported in 2 patients suffering from chronic perennial bronchial asthma of allergic origin.

2. Administration of the hormones produced marked clinical improvement, decrease in circulating eosinophils and increase in the excretion of adrenal cortical metabolites in one of the patients. The response of the second patient to the injection of ACTH, ACTH with ascorbic acid, and epinephrine hydrochloride was weak and transitory.

3. The possible significance of the findings in the pathogenesis of bronchial asthma is discussed.

Acknowledgment

We are very much indebted to Dr. N. O. Calloway and Mr. Robert H. Kyle for their advice and assistance in the determination of adrenal cortical metabolites in the urine. Dr. Robert E. Muehrcke and Mr. James Conklin assisted us by duplicating and thus controlling peripheral eosinophil counts throughout the study. Mrs. Ruth Best of the Illustration Studios, University of Illinois College of Medicine, prepared the figures.

APPENDIX

Case history: T. S.

A 46-year-old colored housewife, a known asthmatic, was admitted for tightness in the chest, wheezing and shortness of breath. The patient stated that she has had bron-

chial asthma since 3 years of age; severe attacks occurred usually during damp weather. She described her attacks as being a tight knot-like sensation in the front of her chest, the breath being short and gasping, accompanied by loud wheezing. She had difficulty in breathing during inspiration as well as expiration. During attacks she was unable to lie down and often spent the night in a chair in order to breathe.

She had been treated by her local physician prior to 1946. Subsequently, she was followed in the Research and Educational Hospitals' Allergy Clinic, where she was found to be allergic to house dust and atmospheric molds. She had been treated with increasing doses of either allergen during the previous three years. Two weeks prior to admission she began to have severe attacks of bronchial asthma, resistant to routine therapy. On admission, wheezing and marked dyspnea were the predominant symptoms.

Physical examination revealed a well-developed, obese, colored female with marked respiratory distress. Her chest showed an increased AP diameter. The lungs showed prolonged expiratory phases and many wheezes as well as musical rhonchi through both inspiration and expiration. Respiration was 28 per minute. The heart was not increased in size and there were no abnormal sounds, and no murmurs. B. P. was 112/76, and the pulse was 60. There was a perforation of the left ear drum but no discharge was noted; the right ear drum was scarred. The results of physical examination were normal in all other respects.

Laboratory data: Urinalysis showed nothing abnormal. The blood count was within normal limits and routine serologic reaction was negative. Fasting blood sugars varied between 64 and 88 mg. per 100 ml., NPN was 30 mg. per 100 ml., cholesterol was 270 mg. per 100 ml., and cholesterol esters were 187 mg. per 100 ml. Serum albumin was 5.1, and globulin 2.3 grams per 100 ml.

Case history: W. P.

A 59-year-old colored, unmarried barber was admitted with a history of chronic perennial bronchial asthma, acute during the past three weeks. He remembered that his first attack occurred thirty years before; since then, he had had frequent severe attacks at regular intervals.

For ten years he had been under observation at the Research and Educational Hospitals of the University of Illinois. He showed positive skin reactions to various inhalants including house dust and atmospheric molds.

His condition had become more severe during the preceding four years. On several occasions, he been brought to the hospital with marked respiratory distress which required injection of aminophylline for relief.

He was studied extensively during the summer of 1948 at the Veterans Administration Hospital at Hines, Illinois.

In September, 1949, he experienced increasing respiratory difficulties but stated on admission in October, 1949, that he felt somewhat better than during the preceding weeks.

The history of the patient revealed that his mother and one sister suffered from bronchial asthma. His personal history was otherwise noncontributory.

The *physical examination* revealed a well-developed, well-nourished patient who was not in acute distress. His chest showed very little excursion with respiration and the AP diameter of the thorax was increased. The lungs were hyper-resonant to percussion, fremitus was normal, the expiration was prolonged and musical high-pitched rhonchi and wheezes were heard throughout the chest on both expiration and inspiration but more so on expiration. The heart was normal to percussion and auscultation. Palpation

of the abdomen showed the liver edge one finger-breadth below the right costal margin. The abdomen was otherwise normal, showing no tenderness or masses. In all other respects, the findings on physical examination were normal.

Laboratory data: Urinalysis showed nothing abnormal. Blood glucose, NPN, cholesterol and cholesterol esters were within normal limits. Serum albumin was 3.7, and globulin 3.2 grams per 100 ml.

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THE METABOLISM OF ETHINYL ESTRADIOL IN MAN*

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IN REVIEWING the literature on ethinyl estradiol, Thompson has pointed out that despite the increasing clinical use of this orally potent estrogen, there is a relative paucity of information on the more subtle details of its fate in the various parts of the body, such as its site of inactivation, degradation products, path of excretion, and effect on the pituitary (1). As part of a study on the metabolism of therapeutic doses of the natural estrogens in man we reported that we were unable to detect colorimetrically, measurable amounts of estradiol, estrone, and estriol in the urine following oral treatment with 1.0 mg. of ethinyl estradiol, whereas a somewhat similar dose (2.0 mg.) of α-estradiol yielded measurable amounts of all three of these estrogens in the urine (2, 3). Apparently no other studies of the urinary estrogen excretion products of ethinyl estradiol have been reported. In view of the important implications of such a study with regard not only to the intermediary metabolism of this derivative of α-estradiol, but to the mode of action of the natural estrogens as well, it seemed of interest to reinvestigate at higher dosage levels and with a more rigorous fractionation procedure (4), the metabolism of ethinyl estradiol in man. The results of 4 of these studies form the subject matter of this report.

EXPERIMENTAL

The 4 men from whom the data herein described were derived, were selected from the urologic service of the Rees-Stealy Clinic because they had been found to excrete in the urine measurable amounts of estrone, estradiol, and estriol following treatment with single therapeutic doses of the natural estrogens, α-estradiol and estrone.[1] Three of the men had been orchiectomized for carcinoma of the prostate; the fourth (JAM) had had a

Received for publication September 23, 1950.

* This work was supported by grants from the National Cancer Institute of the U. S. Public Health Service, and from the American Cancer Society on recommendation of the Committee on Growth.

[1] To be published.

prostatic resection for benign prostatic hypertrophy. All patients were ambulatory and were receiving no other medication at the time the studies were being made.

The ethinyl estradiol was administered in single oral doses, and the forty-eight-hour post-treatment urine collection with toluene as a preservative was started immediately following medication. Equal aliquots of each specimen were hydrolyzed *a*) by boiling under nitrogen for twenty minutes following the addition of 15 vol. per cent of concentrated hydrochloric acid; *b*) by immersion in a boiling water bath for three hours following addition of 15 vol. per cent of concentrated hydrochloric acid and 4 Gm. per cent of zinc dust (5). Each hydrolysate was processed and chromatographed as previously described (6, 7, 8). Additional fractionation of each chromatographic fraction was performed as previously reported (4), *i.e.*, the 2 per cent methanol-benzene chromatographic fraction (estrone) was fractionated by means of Girard's reagent T into nonketonic and ketonic fractions and the 5 per cent methanol-benzene (estradiol) and 30 per cent methanol-benzene (estriol) fractions by means of phthalic anhydride esterification into nonesterified and esterified fractions. Aliquots of each fraction were tested with the Kober reagent (6) and with 90 per cent concentrated sulfuric acid as described by Umberger and Curtis (9).

RESULTS

Data on pure ethinyl estradiol. At room temperature ethinyl estradiol forms a red color immediately on contact with the undiluted Kober reagent. This is in marked contrast to the natural estrogens (α-estradiol, estrone, estriol) which, on heating, produce an initial yellow color with the undiluted Kober reagent. β-Estradiol produces the initial yellow color at room temperature (10). Heating at 100°C for two minutes and dilution after cooling with dilute sulfuric acid (20 per cent v/v) did not materially alter the character of the Kober color (Fig. 1). The ethinyl estradiol Kober color product (Fig. 1) also showed (Beckman DU spectrophotometer) a maximum absorption at 540 millimicrons in contrast to 520 mμ maxima for the natural estrogens. The ultraviolet absorption curve of ethinyl estradiol in 95 per cent ethyl alcohol (230 to 300 mμ) was typical of published (11) curves for estrone, α-estradiol, and estriol.

On heating ethinyl estradiol with 90 per cent sulfuric acid and scanning the color between 400 and 600 mμ according to the method of Umberger and Curtis (9), a spectral absorption curve was obtained (Fig. 2) which clearly differentiated ethinyl estradiol from the natural estrogens, since its maximum at 520 to 530 mμ was well removed from the maxima (430 to 480 mμ) exhibited by the natural estrogens. Furthermore, the color developed at room temperature. In our liquid chromatogram technique for

the fractionation of estrone, α-estradiol, and estriol (7), ethinyl estradiol appeared in the 5 per cent methanol-benzene (estradiol) eluate and consequently could not be separated from estradiol by this technique.

Conditions (heating for three hours at 100°C with a pyridine solution of phthalic anhydride (4)) which yielded quantitative esterification of α-estriol with phthalic anhydride to form hemiphthalates, caused no esterifi-

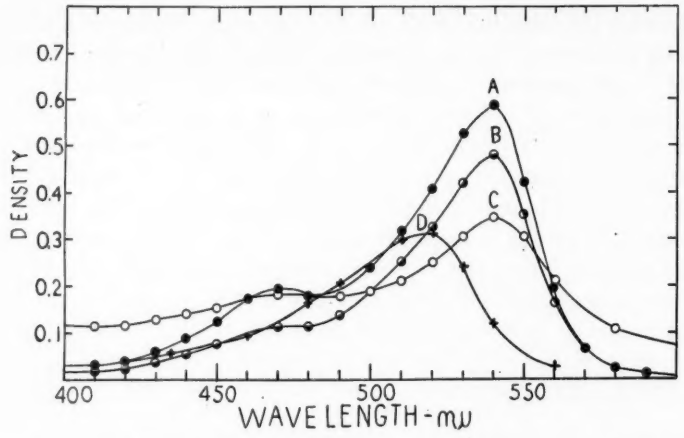

FIG. 1. Absorption spectra of colors formed in the Kober reaction. (A) 100 micrograms of ethinyl estradiol heated for two minutes at 100° C. with 3 ml. of undiluted Kober reagent and diluted to 10 ml. final volume with 20 per cent sulfuric acid. (B) 100 micrograms of ethinyl estradiol at room temperature for two minutes with undiluted Kober reagent. (C) Aliquot of nonesterified moiety (ethinyl estradiol) of F-2 chromatographic fraction from urine of man following treatment with ethinyl estradiol—at room temperature with undiluted Kober reagent. (D) 100 micrograms of α-estradiol treated by Kober reagent in conventional two-stage procedure (6).

cation of ethinyl estradiol presumably due to stearic hindrance effects. Advantage has been taken of this observation to separate α-estradiol and ethinyl estradiol, since the hemiphthalate of α-estradiol may be removed from its ethyl ether solution by aqueous potassium carbonate (4) under conditions which retain unesterified ethinyl estradiol in the ether phase.

Colorimetric urinary estrogen excretion data following ingestion of ethinyl estradiol in men. Although our 3 chromatographic fractions F-1 (2 per cent methanol-benzene), F-2 (5 per cent methanol-benzene), and F-3 (30 per cent methanol-benzene) gave rise to 6 fractions subsequent to application of Girard's reagent-T and phthalic acid esterification, the colorimetric data from 2 of these (F-1 nonketonic and F-3 nonesterified) were of no significance and were omitted from the table (Table 1). It is to be emphasized that the color correction equation (8) which was employed in calculating

the estrogen titer of each estrogenic fraction introduces an uncertainty factor of ±7 micrograms of estrone, ±10 micrograms of estradiol, and ±5 micrograms of estriol per twenty-four-hour specimen and accounts for the negative values listed in the table.

TABLE 1. COLORIMETRIC (KOBER TEST) URINARY ESTROGEN EXCRETION DATA ON MEN AFTER INGESTION OF ETHINYL ESTRADIOL

(48-hour post-treatment urine)

Case and amount of Ethinyl Estradiol	Aliquot	Hydrolysis	Estrogen titer (micrograms per 24-hour aliquot)			
			Chromatographic fraction			
			F-1 (2% M-B) Girard's T ketonic	F-2 (5% M-B) Phthalic anhydride		F-3 (30% M-B) Phthalic anhydride esterified
				nonesterified	esterified	
			Estrone	Ethinyl Estradiol	Estradiol	Estriol
1 (JRB) 5 mg.	A	Simple	2	57	−5	2
	B	Zn-HCl	0	46	2	7
2 (FMB) 10 mg.	A	Simple	0	49	−10	0
	B	Zn-HCl	0	88	−10	5
3 (JAM) 10 mg.	A-1	None	1	10	2	0
	A-2	Autoclave (15 lbs.) at pH 1 for 1 hr.	−1	29	0	−5
	B	Zn-HCl	−2	148	−5	27
4 (REB) 10 mg.	A-1	None	1	69	0	0
	A-2	Simple (10% HCl)	4	80	−6	3
	B	Zn-HCl	−4	159	−6	8

In addition to simple hydrochloric acid hydrolysis of the urinary estrogens we have included data on aliquots to which zinc dust was added during hydrolysis, since it has been shown that an unidentified urinary me-

tabolite of 16-ketosterone and 16-keto-α-estradiol gives rise to Kober color products only when zinc dust is added during hydrochloric acid hydrolysis (12). Furthermore, the addition of zinc dust has been found to eliminate effectively urinary pigments which occasionally arise from simple acid hydrolysis of urine and appear in the nonesterified moiety of the F-2 (estradiol) chromatographic fraction (4).

Although the ingestion of 2.0 mg. of α-estradiol by men has yielded measurable amounts (10 to 20 per cent) of estrogen (estradiol, estrone, estriol)

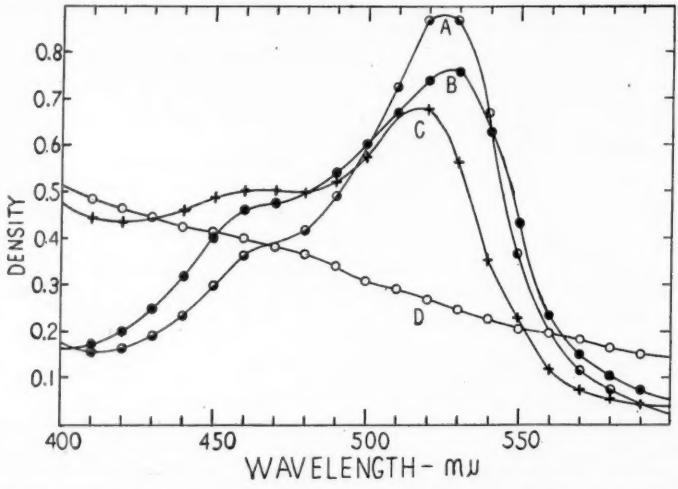

FIG. 2. Absorption spectra of colors produced with 5 ml. of 90 per cent sulfuric acid. (A) 55 micrograms of ethinyl estradiol at room temperature for twelve minutes. (B) 55 micrograms of ethinyl estradiol at 100° C. for twelve minutes. (C) Aliquot of the nonesterified moiety (ethinyl estradiol) of F-2 chromatographic fraction from urine of man following treatment with ethinyl estradiol. (D) Aliquot of the nonesterified moiety of the F-2 chromatographic fraction from urine of man without treatment.

in the urine (3), as much as 10 mg. of ethinyl estradiol yielded no detectable amounts of any of these estrogens above the level commonly observed to be of endogenous origin. However, in all 4 cases, a product was yielded in the F-2 (estradiol) chromatographic fraction which was nonesterifiable with phthalic anhydride under conditions which quantitatively esterify α-estradiol. This residue yielded at room temperature an immediate pink color with the undiluted Kober reagent. The spectral absorption curve of this Kober color product (Fig. 1) as well as that produced by 90 per cent sulfuric acid in the Umberger and Curtis reaction (Fig. 2) was similar to those exhibited by ethinyl estradiol when similarly treated. Since ethinyl estradiol which was added to suitable urinary residues after hydrolysis also

appeared in the nonesterified moiety of our F-2 chromatographic fraction,[2] these data strongly suggest that the exogenous ethinyl estradiol was excreted in part unchanged except possibly for conjugation.

To test further the possibility of conjugation of the excreted hormone, aliquots of urine, one from Case 3 and one from Case 4, were first extracted with ethyl ether and this extract as well as a similar one made following simple hydrolysis, was separately analyzed for estrogen content. In each of these 2 cases the major portion of the putative ethinyl estradiol appeared in the hydrolyzed fraction.

The maximum recovery of ethinyl estradiol in Cases 3 and 4 was elicited by zinc dust hydrochloric acid hydrolysis and averaged approximately 3 per cent of the ingested hormone. Perhaps the urine collection periods might profitably have been extended beyond the forty-eight hours but recovery of exogenous estrogen beyond this period usually is negligible (13).

DISCUSSION

The ester derivatives of the natural estrogens, for example the 3-monobenzoate and 3-17 dipropionate of α-estradiol are characterized by protracted, persistent, estrogenic action perhaps due to the slow release of the hormone to the general circulation. These ester derivatives, however, undergo the same interconversions, destruction, and elimination in man as have been found for the free estrogens (2 and 13–17). A great many other derivatives and transformation products of the natural estrogens have been prepared and assayed, but no highly potent compound other than 17-ethinyl estradiol has been encountered (18). No satisfactory explanation for the high estrogenic potency of this compound has been suggested. Our failure to find measurable amounts of the natural estrogens in the urine of the 4 men following treatment with ethinyl estradiol suggests that the estrogenic activity is inherent in the compound itself rather than due to its transformation into the natural estrogens. Segaloff (19) has reached a similar conclusion. Although man apparently does not possess the capacity to convert ethinyl estradiol to the natural estrogens, its transformation (inactivation) perhaps at other points in the molecule is indicated by the relatively low recovery (3 per cent) of the administered hormone. On the other hand, these studies demonstrate that man is capable of conjugating ethinyl estradiol *in vivo* and that the hormone and its conjugate are eliminated in part in the urine.

These observations should stimulate further investigation of the adequacy of the ethinyl estradiol to perform all the functions of the natural estrogens, since the possibility that estrone and estriol play some unique

[2] Unpublished data.

role in mediating the estrogenic response in women, has not been excluded. For example, there is experimental evidence that estriol or its complex may be the active circulating hormone (20).

The apparent inability of man to remove the ethinyl group from ethinyl estradiol *in vivo* commends the radioactive analogue of this estrogen derivative as a suitable compound with which to follow the intermediary metabolism of the natural estrogens. One such radioactive estrogen (radioactive 17-methyl estradiol) has been utilized for this purpose (21).

SUMMARY

We have been unable to detect measurable amounts of estradiol, estrone, and estriol in the urine of men following ingestion of a single 10-milligram dose of ethinyl estradiol. There was evidence (colorimetric) of recovery of unaltered (except for conjugation) ethinyl estradiol in the urine, which did not exceed 3 per cent of the administered dose.

Acknowledgment

We wish to thank Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc., for making available to us special 5-milligram capsules of ethinyl estradiol for use in this study.

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STUDIES ON THE CONVERSION OF TESTOSTERONE TO URINARY 17-KETOSTEROIDS IN NORMAL HUMAN MALES AND MALES WITH HEPATIC AND OTHER DISEASES*

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ACCORDING to available evidence the urinary 17-ketosteroids are derived solely from steroids of the adrenal cortex and testes. It has been assumed that changes in the amounts of the 17-ketosteroids in urine reflect alterations in function of these glands. However, this view oversimplifies the picture and interpretations of data on urinary 17-ketosteroids must remain uncertain so long as the factors which influence the metabolism of their precursors remain largely unknown.

Although many different 17-ketosteroids (1) have been isolated from normal and abnormal urines, it appears that the 17-ketosteroids found in largest amounts in normal human male and female urines under usual circumstances of analysis are androsterone and etiocholan -3(α)-o1-17-one. That they are derived from the adrenal cortex is clear but it is unknown whether the adrenal cortical precursors are, in the main, androgenic, 19-carbon steroids (possibly, Δ^4-androstene-3, 17-dione or even dehydroisoandrosterone) or native 21-carbon pregnenes. As yet there is no good evidence that the 11-oxygenated pregnenes give rise to androsterone or etiocholan-3(α)-o1-17-one. However, in man these common urinary 17-ketosteroids are the major urinary derivatives of the 19-carbon testicular androgen, testosterone. Although there is no evidence to indicate that human "adrenal androgen" is or is not testosterone itself, it is still possible that testosterone or its metabolites are subject, at least in part, to the same metabolic influences as the unknown adrenal cortical precursor and undergo similar degradation to 17-ketosteroids. It has been demonstrated that Δ^4-androstene-3, 17-dione, which has been isolated from adrenal

Received for publication September 15, 1950.

* Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine and by a contract between the Office of Naval Research, Department of the Navy and Yale University (NR 113–018)

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tissue, is formed when testosterone is incubated with liver slices (2); also, administration of Δ^4-androstene-3, 17-dione produces a rise in 17-ketosteroid excretion (3), and androsterone and etiocholanolone have been isolated in urine after its administration (4). Similar excretory products are formed from dehydroisoandrosterone (5), a steroid which undoubtedly arises from adrenal activity in man although it has not been isolated from the adrenal tissue of animals. Mainly on the basis of clinical observations it has been felt by some investigators that the human adrenal elaborates steroids which have strong anabolic and androgenic properties like testosterone, and that these steroids are distinct from those 21-carbon pregnenes which mainly affect protein and carbohydrate metabolism (6). For these reasons and because the precise adrenal precursor of the commonest urinary 17-ketosteroids is still unknown, it seemed reasonable to investigate certain factors which might influence the degradation of the androgen, testosterone. Naturally, the findings pertain only to testosterone, but the possibility that they may pertain, at least in part, to the adrenal cortical precursors of the urinary 17-ketosteroids must be considered.

The extent of conversion of testosterone to 17-ketosteroids can be estimated in a short-term experiment, wherein a single, rather large dose of testosterone is administered and the acute rise in urinary 17-ketosteroids is measured. Such an acute experiment eliminates to a considerable degree the problem of varying rates of endogenous hormone production which are difficult to estimate and control in experiments wherein exogenous hormone is administered for prolonged periods. Therefore, in these studies, immediately after obtaining control specimens of urine, 50 mg. of testosterone propionate[1] was administered and the urine was collected for the subsequent forty-eight hours.

In this manner the metabolic conversion of testosterone to 17-ketosteroids was studied in normal men, in patients with severe parenchymatous liver disease, and in patients who were ill from a variety of disorders other than hepatic disease. (The response of normal women in different phases of the menstrual cycle is reported in a separate paper (7).) From an analysis of these data it was hoped to determine whether the low urinary 17-ketosteroid excretion seen in patients who are ill from a variety of diseases or from liver damage could be related to an altered rate of metabolism of a model precursor hormone; also, whether the widely different amounts of 17-ketosteroids excreted by apparently normal men could be similarly related. It has been demonstrated that rat (8) and rabbit (2) liver tissue is active in the *in vitro* destruction of testosterone. The importance of the liver in the conversion of testosterone to 17-ketosteroids is not entirely clear (9). However, since different species of animals metabolize and ex-

[1] The testosterone propionate (Perandren) was kindly supplied by Ciba Pharmaceutical Products, Summit, New Jersey, through the courtesy of Dr. F. E. Houghton.

crete steroid hormones in different ways, it seems necessary to determine, so far as possible, the role played by the human liver in these processes.

MATERIALS AND METHODS

To determine the control level of 17-ketosteroid excretion, a 24-hour specimen of urine was collected immediately before the injection of testosterone propionate. In order to determine, under our own experimental conditions, the variability of values for control urines in the same individual, two consecutive control 24-hour collections were made on 23 subjects. As has been observed by others (9, 10) the day-to-day variation in 17-ketosteroid excretion is relatively small in the same individual. The average difference between the two 24-hour urines was only 2.1 mg. (range: 0.0 mg. to 5.1 mg.) in these 23 individuals. Thus for the remainder of the studies it seemed reasonable to utilize only one accurately collected 24-hour specimen as the control urine.

Within one and one-half hours after the completion of this control collection, 50 mg. of testosterone propionate was injected intramuscularly, always by the same individual, into the upper outer quadrant of the buttocks of the subjects. Accurate urine collections were continued for the next forty-eight hours. All subjects were questioned as to the accuracy of the collections. All urines were preserved with 5 ml. of concentrated hydrochloric acid and stored in the icebox until extracted and assayed. Most of the urines were analyzed within four days of collection; none was stored more than ten days.

The 17-ketosteroid content of the urine was estimated by application of the Zimmermann reaction (11) to the total neutral steroid fraction. An aliquot of the urine specimen was refluxed for ten minutes, after addition of 10 per cent by volume of the concentrated HCl, and extracted with ether. The ether extract was washed with saturated $NaHCO_3$ solution and 4 per cent aqueous KOH. After evaporation to dryness under diminished pressure with the aid of absolute alcohol, the residue from the washed ether extract was dissolved in 95 per cent alcohol from which aldehydes had been removed by redistillation with 2, 4-dinitrophenylhydrazine. The colorimetric determination was carried out according to the technique of Holtorff and Koch (12) with two modifications. The digestion time was increased to 105 minutes (13) and 60 per cent rather than 95 per cent alcohol was used for dilution of the reaction mixture (14). Androsterone (international standard or a crystalline preparation of equivalent purity) was used as the standard, and the color values obtained from urine extracts were corrected by deduction of the blank suggested by Holtorff and Koch.

The extent to which testosterone was converted to urinary 17-ketosteroids was calculated from the increment (in milligrams) in the output of 17-ketosteroids which occurred in forty-eight hours after the injection, and was expressed as per cent of injected testosterone (41.8 mg. of testosterone in 50.0 mg. of testosterone propionate).

By this means data were obtained on 21 normal males concerning the extent to which testosterone was converted to neutral 17-ketosteroids; the

experiment was repeated in 3 individuals, making a total of 24 experiments on normal subjects. Similar studies were performed on 8 male patients who were seriously ill from a variety of diseases (*see* Table 1); in none, however, was organic liver disease believed to be present. Similarly studied were 5 males and 3 females with diffuse, organic liver disease (*see* Table 1), which was almost uniformly of a severe degree. Four other patients were

TABLE 1

Patient	Age (yrs.)	Clinical diagnosis	BSP*	Serum bilirubin (mg. per-cent)		Prothrombin time† (per cent normal)	Cephalin flocc. (48 hrs.)	Serum‡ (Gm. per cent)	
				Direct	Indirect			Albumin	Globulin
			Acutely ill males						
P.J., C-29728	25	Ulcerative colitis, active. Active rheumatoid arthritis.	6	0.11	0.66	No clinical evidence of hepatic disease			
P.B., 61935	57	Active pulmonary tuberculosis. Carcinoma of tonsil. Malnutrition, severe.	—	—	—	No clinical evidence of hepatic disease			
J.P., A-48232	41	Acute rheumatic fever with polyarthritis and carditis.	—	—	—	No clinical evidence of hepatic disease			
E.V., C-36426	40	Bronchiectasis, severe. Pansinusitis, active. Malnutrition. Chronic cor pulmonale.	3	0.02	0.19	No clinical evidence of hepatic disease			
J.L., C-36013	75	Malnutrition, severe. Pulmonary emphysema and fibrosis.	—	—	—	No clinical evidence of hepatic disease			
L.A., A-35575	*60	Cellulitis of foot, severe. Diabetes mellitus. Hypertensive vascular disease.	—	—	—	No clinical evidence of hepatic disease			
R.T., B-84497	33	Tuberculous meningitis.	—	—	—	No clinical evidence of hepatic disease			
M.L., B-26256	69	Acute cystitis, severe, with fever (103°). Hemorrhage from duodenal ulcer 5 days before study.	1	0.02	0.19	100	1+	3.03	2.4
			Males with diffuse hepatic disease						
C.V., C-5673	35	Portal cirrhosis, severe. Alcoholism, chronic. Malnutrition.	16	0.41	0.66	64	4+	3.5	3.5
T.S., B-89399	56	Portal cirrhosis, moderate. Malnutrition.	20	0.38	0.99	—	3+	2.3	4.2
A.R., B-53523	37	Portal cirrhosis, severe. Alcoholism, chronic.	—	2.23	5.36	45	4+	1.72	5.19
B.D., B-36159	54	Portal cirrhosis, severe. Alcoholism, chronic.	—	3.84	7.29	29	4+	1.61	4.97
W.N., B-51358	62	Marked hepatomegaly secondary to Hodgkin's disease.	10.5	0.22	0.56	—	4+	1.83	4.34
			Convalescent males						
C.B., C-33245	40	Convalescent from poliomyelitis, 3 mos. previously. Muscle atrophy, arms and legs.	—	—	—	No clinical evidence of hepatic disease			
J.R., C-33930	28	Convalescent from poliomyelitis, 3 mos. previously. Muscle atrophy, arms and legs.	—	—	—	No clinical evidence of hepatic disease			
E.C., B-98-54	47	Old rheumatoid arthritis, and "burned out" spondylitis. Bilateral cataracts.	—	—	—	No clinical evidence of hepatic disease			
R.W., B-37445	59	Mild hemorrhage from superficial gastritis 6 days before study. No further bleeding.	—	—	—	No clinical evidence of hepatic disease			
			Females with diffuse hepatic disease						
M.S., C-28204	36	Portal cirrhosis, severe. Fever (? cause), Alcoholism, chronic.	25	0.40	0.80	50	4+	2.0	3.92
M.C., B-91258	40	Portal cirrhosis, severe. Malnutrition.	44	0.48	1.18	—	3+	1.66	4.55
M.D., C-25426	27	Hepatitis, severe, probably serum-transmitted. Hepatic coma and death 2 weeks after study.	63	3.57	7.19	27	4+	3.64	3.03

* Measured as per cent bromsulfalein retained in serum 45 minutes after injection of 5 mg. of bromsulfalein per Kg. of body weight.

† Prothrombin time determinations were carried out by Quick's one-stage method using a thromboplastin suspension, with controls for each determination. As customary, the percentages signify the per cent of prothrombin concentration compared to normal control concentrations.

‡ Serum proteins were determined by the method described by Milne, J.: *J. Biol. Chem.* **169**; 595, 1947.

studied who were in the late phase of convalescence from acute disease and who were in fairly good general health at the time of study.

RESULTS

The variations in the extent to which normal men convert testosterone to urinary 17-ketosteroids are shown in Figure 1. On an average (mean), 35 per cent of the injected testosterone (41.8 mg. testosterone, equivalent to 50 mg. of the propionate) was excreted as urinary 17-ketosteroids within forty-eight hours of its administration. The rate of conversion, however, was surprisingly varied in these normal individuals, ranging from 9.6 to 71 per cent. In 3 instances the experiments were repeated and, even in the same individual, the extent of transformation to 17-ketosteroids was considerably different at different times (*e.g.*, 58.5 and 71 per cent in D. S., 9.6 and 36.6 per cent in P. M., and 33.1 and 19.6 per cent in W. E.). In other analyses of these data no significant correlation was found to exist between the weights of these subjects and their control excretion of 17-

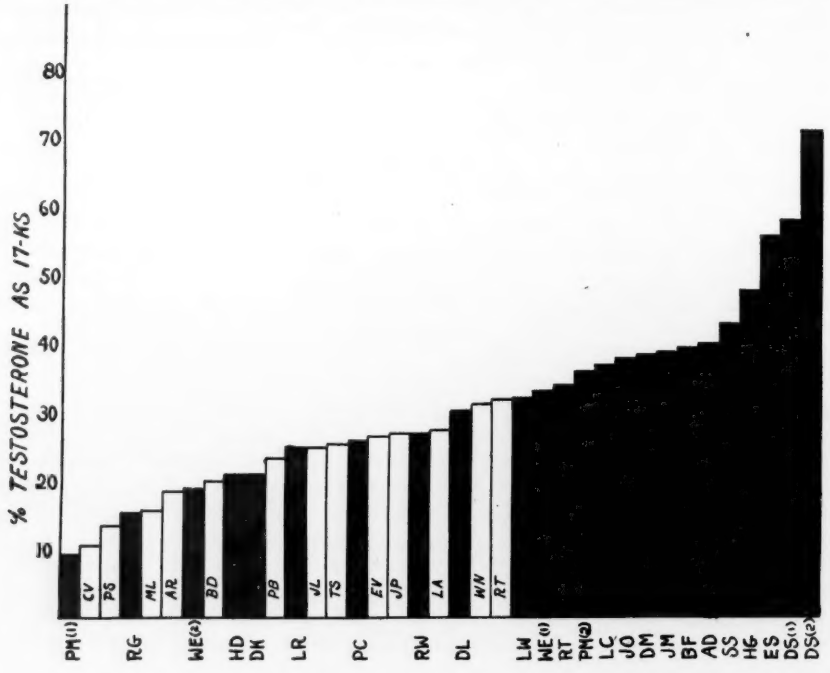

FIG. 1. Distribution of subjects according to their ability to transform 50 mg. of testosterone propionate (41.8 mg. of testosterone) to urinary 17-ketosteroids. The solid bars represent normal, healthy males; the open bars represent males who were acutely ill with hepatic and other disorders. The nature of these disorders is summarized in Table 1.

ketosteroids or between weight and the ability to metabolize a uniform amount of testosterone.

In view of the wide range in the excretion rate of 17-ketosteroids in normal individuals, an effort was made to determine whether or not this control level was related to the extent to which testosterone could be converted to 17-ketosteroids. These data are summarized in Figure 2 (normal males: solid dots). The scatter of points around the mean (35 per cent) is such as

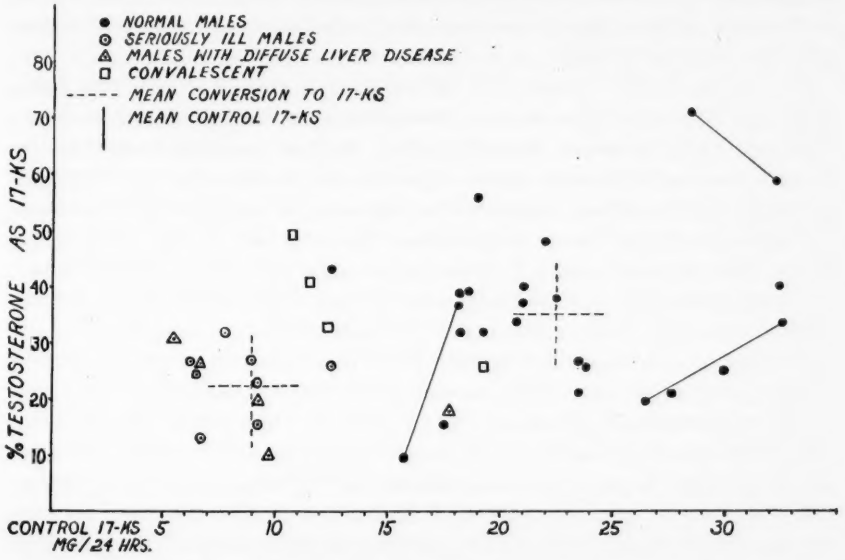

FIG. 2. The relation between the control level of excretion of 17-ketosteroids to the extent of conversion of testosterone. All of these subjects are males. The dotted cross on the right pertains to normal, healthy males; on the left, to the seriously ill patients and those with hepatic disease. The solid lines join the points obtained on two different studies on the same individuals (P. M., D. S. and W. E.).

to preclude a significant correlation,[2] ($r = \pm 0.01$). Also, in order to test this effect at a different dosage level, 75 mg. of testosterone propionate was given to 2 normal males who were of similar weight (148 and 140 pounds) but who had widely divergent control levels of 17-ketosteroid excretion; again, there was no tendency by the individual with the high control level of steroid excretion to transform more testosterone to urinary steroids.

Five males and 3 females with diffuse, parenchymatous liver disease were similarly studied. Some pertinent clinical data on these patients are summarized in Table 1. The facility whereby the male patients convert testosterone to 17-ketosteroids is shown in Figures 1 and 2; in these, from

[2] r = coefficient of correlation.

10.5 per cent to 31.4 per cent of the steroid was thus transformed within forty-eight hours of injection. All of the values fell below the mean of 35 per cent for normal men, yet none fell below the range of normal for men or women (7). Data on the 3 women are not included in the figures but these patients showed a considerable ability to transform testosterone to 17-ketosteroids. M. S. converted 16.8 per cent of the testosterone to urinary 17-ketosteroids in twenty-four hours, and M. C. and M. D. converted 24.2 and 21.6 per cent in forty-eight hours, respectively. Patient M. D. had such severe, diffuse hepatic disease that she died in hepatic coma two weeks after the study was made.

In addition to the 5 males with diffuse liver disease, 8 seriously ill males were also studied but in none of these was liver disease apparent. From Figures 1 and 2 it is seen that the ability of these patients to metabolize testosterone to 17-ketosteroids is impaired only to the same minor extent as patients with diffuse, organic liver disease. All had low control values for 17-ketosteroids; none metabolized testosterone to an extent that reached the normal, mean conversion rate (35 per cent). On this basis, these patients could not be differentiated from those with hepatic disease. Also on the same basis none of these 13 male patients (those that had hepatic disease and those that were ill from other disorders) converted testosterone to 17-ketosteroids to an extent that was outside the wide normal range of conversion, although on an average these patients converted somewhat less than normal. Their mean conversion rate was 22.5 per cent, as compared to 35 per cent for normal males. Further, all of the conversion rates for these ill patients lay below 32.2 per cent. The means of the percentage conversion values for the patients and the normal subjects were compared by the *t*-test (15) and found to be significantly different at the one per cent level, *i.e.*, there is less than one chance in a hundred that the groups were not different in this respect. An analysis of variance and application of the *F*-test confirmed this conclusion and revealed that the variance as well as the means of the two groups were significantly different Both of these differences are obvious on inspection of Figures 1 and 2.

Although there is this significant difference in the extent of transformation of testosterone to urinary product by seriously sick patients and normal individuals, the 4 patients who were not acutely ill but in a late stage of convalescence converted 25.8, 33, 42.2 and 49 per cent of the exogenous testosterone to 17-ketosteroids, values entirely similar to those for normal men.

DISCUSSION

In earlier studies wherein testosterone or its propionate was administered over a considerable period of time to normal and eunuchoid males

it was observed that the titer of biologically active androgen in the urine increased (16, 17, 18). Without knowledge of the relative quantities of the various urinary 17-ketosteroids (some biologically active and other inactive) it was not possible to calculate accurately the extent to which testosterone was converted to these substances. If it was assumed that the major portion was androsterone (an assumption not wholly valid), then from 13 to 46 per cent could be accounted for as urinary steroid (16). The present investigation on normal males indicates a similar wide variation in the extent of conversion of testosterone to total urinary 17-ketosteroids; from 9 to 71 per cent of the testosterone appeared as these urinary products.

It has been continually puzzling why apparently normal individuals exhibit wide differences in their control rates of excretion of 17-ketosteroids. That the adrenal cortices of one normal male elaborate more than twice as much precursor hormone as do those of another is possible, but this explanation has not been entirely satisfying. It was hoped that a study of the metabolism of testosterone would reveal differences in the rate of its metabolism in different individuals to account, in part, for this baffling situation. Yet no correlation was found between the control level of excretion of 17-ketosteroids by different individuals and the textent to which they formed 17-ketosteroids from testosterone. Individuals with low control 17-ketosteroid excretion were as likely to convert a high proportion of the administered testosterone propionate to 17-ketosteroids as those with high control values. Also, considerable difference in the extent of this conversion was exhibited by the same individual at different times. It is not clear what these findings in normal men truly signify, since the excretion of 17-ketosteroid varies little from day to day and the level is generally characteristic for the individual. If our results on this particular metabolic process are characteristic also of the adrenal precursors of the urinary 17-ketosteroids, then it would seem possible that the rate of formation of precursor hormone by the adrenal is considerably different in different individuals and may vary considerably in the same individual from time to time. However, until more complete data are available on the nature of the adrenal hormones in man, this interpretation must remain only a tentative one.

The site of transformation of precursor steroids to the steroids which appear in urine as 17-ketosteroids is not known. From studies with hepatic tissue of animals it seems that the liver may be active in the partial degradation of testosterone but that the site of formation of the major urinary 17-ketosteroids (*e.g.*, etiocholanolone and androsterone) remains obscure (2, 8, 18). In the studies herein reported it is apparent that humans with diffuse hepatic disease are able to transform testosterone to 17-ke-

tosteroids to an extent that is still within the wide range for normal rates of conversion. However, all of the patients converted amounts which were less than the mean for normal individuals. Liver damage to the extent present in these patients, therefore, partially impairs this metabolic process but the impairment is far from complete. If the human liver is still all-important for these transformations it must be extremely efficient in carrying out these reactions. If not, other sites must be considered. As a test of liver function, the measurement of the extent of conversion of testosterone to urinary steroids would be particularly insensitive, mainly because normal individuals possess such a varying ability to bring about the conversion. However, in a study of 4 males with cirrhosis, Lloyd and Williams (20) found no increase in the urinary 17-ketosteroids after the administration of 50 mg. of testosterone propionate. Our studies do not confirm this finding.

Males who were acutely and severely ill from disorders other than frank hepatic disease also revealed an ability to transform testosterone to 17-ketosteroid still within the range, but invariably below the mean for normal individuals. Thus, they could not be differentiated from those with hepatic disease. All of the 13 points on Figure 2 for the sick males are located below the mean for the 21 normal males. This is a significant difference. This finding points to the possibility that a low excretion of 17-ketosteroids in individuals who are ill from a variety of disorders may not entirely imply a depressed rate of precursor hormone formation but rather that the disposition of these precursors is diverted to channels other than to the urinary 17-ketosteroids. To what purpose this diversion might be directed and to what extent, if any, these findings with testosterone apply to the adrenal steroids, is unknown. However, in another type of study by Forbes *et al.* (21) a similar phenomenon may have occurred, although it was not commented upon by the authors. Their patient (figure 6 in the paper by Forbes (21)) was treated with 25 mg. of testosterone propionate daily for nine days prior to elective surgery. As expected, the excretion of 17-ketosteroids rose above control levels while the patient was receiving testosterone; however, after operation, in spite of continuous administration of testosterone, the 17-ketosteroid output fell to levels that were far below even the control levels of excretion. Although this phenomenon could possibly be accounted for by a marked depression of production of endogenous precursor hormones during this phase, an altered metabolism of these substances and of the exogenous testosterone may have been a contributing factor.

SUMMARY

The extent of conversion of testosterone to total urinary 17-ketosteroids was studied in normal males, in males who were acutely ill and in patients

with severe liver damage. Normal individuals exhibited wide differences in their ability to transform testosterone to 17-ketosteroids; from 9.6 to 71 per cent (mean: 35 per cent) of the steroid was thus metabolized. No correlation could be found between the control level of excretion of 17-ketosteroids and the extent of this transformation. Also, considerable difference in the extent of transformation of testosterone to 17-ketosteroids was seen in the same individual at different times. It is not known what these findings signify either in terms of testosterone metabolism or in terms of adrenal cortical function.

In spite of severe, diffuse liver disease considerable testosterone (from 9 to 31 per cent) was converted to urinary 17-ketosteroids. The results could not be distinguished from those obtained on seriously ill patients not suffering from recognizable hepatic disease. The human liver can not be considered to be all-important in the metabolic transformation of testosterone to 17-ketosteroids. However, the observation that the mean extent of this transformation by patients who were ill from a variety of disorders was significantly less than normal indicates that these states can influence the conversion of precursor hormone to excretory product. More data are needed to clarify the nature and extent of this difference. Some theoretical considerations which impinge on these observations are presented.

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STUDIES ON THE METABOLISM OF TESTOSTERONE IN NORMAL WOMEN IN DIFFERENT PHASES OF THE MENSTRUAL CYCLE*

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THAT the size and function of the adrenal cortex can be influenced by ovarian activity has been demonstrated in rats (1–4). It is not known whether adrenal cortical activity in man similarly varies with ovarian activity in any consistent manner. Although the urinary 17-ketosteroids reflect at least certain aspects of adrenal function, certainly no regular alterations in the rate of their excretion have been noted in normal women during different phases of the menstrual cycle (5–9). However, Koets (10) has made the interesting observation that in women suffering from simple hirsutism there does exist a cyclic variation in the 17-ketosteroid excretion correlated with the menstrual cycle. Why the output of 17-ketosteroids should vary in this manner in these women and not in normal women requires explanation. As an approach to an understanding of the relation between ovarian and adrenal cortical function it seemed important to investigate the manner in which a precursor of the 17-ketosteroids is metabolized during different phases of the normal menstrual cycle. Consequently, this report deals with the extent to which normal women, in an acute study, convert a precursor hormone, testosterone, to 17-ketosteroids.

It is not known whether testosterone or a 19-carbon steroid closely related to it, is normally elaborated by the human adrenal. Because testosterone is transformed mainly to androsterone and etiocholan-3(α)-ol-17-one and because of the fact that these substances are the 17-ketosteroids which are excreted in greatest quantity as a result of normal adrenal cortical activity, it seemed reasonable to utilize testosterone as a model compound for this investigation. In fact, without knowledge of the chemical nature of the adrenal cortical precursors of the major urinary 17-

Received for publication September 15, 1950.

* Aided by a grant from the James Hudson Brown Memorial Fund of the Yale University School of Medicine and by a contract between the Office of Naval Research, Department of the Navy, and Yale University (NR 113-018).

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ketosteroids, it is not possible to select the ideal compound for study in this regard, even were it available in quantity. Other observations relating to this problem are discussed elsewhere (11). Though testosterone itself normally may not be elaborated by the adrenal cortices of women or men, a study of the factors which influence its metabolism may still shed light on at least a part of the metabolic pathway taken by the true adrenal cortical precursors as they are transformed into the major urinary 17-ketosteroids. If not, the study will provide information on the influence of ovarian activity on the transformation of an androgen to 17-ketosteroids.

MATERIAL AND METHODS

Eight normal, ambulant women were studied; all were nulliparae; all were in the third decade of life; all had normal, regular menstrual cycles; and all were engaged in scientific pursuits so that their cooperation in obtaining accurate collections of urine was excellent. The technic of studying the acute metabolism of testosterone was identical with that described in a previous paper (11). All of the control collections of urine were made immediately prior to the intramuscular injection of 50 mg. of testosterone propionate.[1] The increase in the urinary 17-ketosteroids in the next forty-eight hours was probably a product, in large part at least, of the exogenously administered testosterone which had been converted to 17-ketosteroids. In 7 of the 8 subjects the test was made twice, once in the luteal phase of one cycle and again in the follicular phase of another. The other subject (Y.O.) was studied only in the luteal phase. A ninth individual (F.R.) unfortunately was studied while convalescent from infectious mononucleosis. Although the experiment was done in the expected "luteal" phase, the anticipated menstruation was nine days "late" and unusually small in amount. This may have been an anovulatory period.

RESULTS

The data on the extent of conversion of testosterone to urinary 17-ketosteroids in normal women during different phases of the menstrual cycle are summarized in Figure 1. It is noted that in all 7 normal individuals who participated in the study during both phases of the cycle, more testosterone was converted to 17-ketosteroids in the follicular than in the luteal phase of the cycles. Even the eighth individual (Y.O.), for whom no data are available in the follicular phase, metabolized less testosterone to 17-ketosteroids in the luteal phase than any of the other normal subjects metabolized during the follicular phase. The average fraction of testoster-

[1] The testosterone propionate (Perandren) was kindly supplied by Ciba Pharmaceutical Products, Summit, New Jersey, through the courtesy of Dr. F. E. Houghton.

one converted to urinary 17-ketosteroids by all of these subjects (excluding F.R.) was 27.2 per cent during the luteal phase and 50.3 per cent during the follicular phase. Although the number of normal subjects involved was relatively small, there were no exceptions to the finding and an analysis

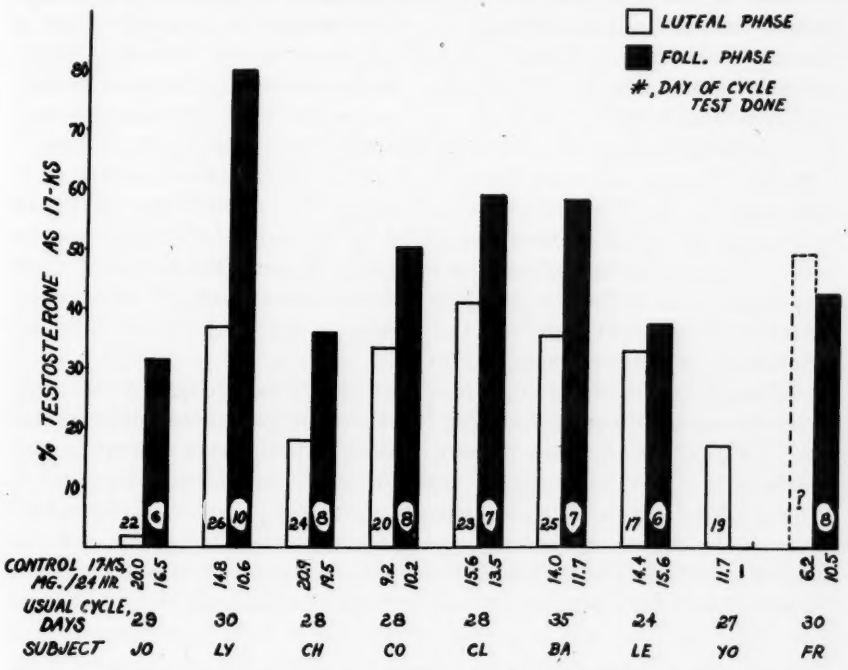

FIG. 1. Summary of data obtained on the extent to which testosterone is metabolized to 17-ketosteroids in normal women in different phases of the menstrual cycle.

of variance of the paired observations and application of the F- test shows that the difference in the percentage of testosterone converted in the two phases of the menstrual cycle was highly significant ($P = <0.005$). It may also be noted that although the control values for 17-ketosteroids in 5 of the 7 normal individuals were slightly higher in the luteal phase than in the follicular, statistical analysis showed the difference to be insignificant. The mean difference was only 1.4 mg. per twenty-four hours.

As noted above, the study on F.R. was complicated by the fact that the test in the "luteal" phase was made while the subject was convalescent from infectious mononucleosis. That she had not fully recovered from her illness is suggested by the unusually low control excretion of 17-ketosteroids; therefore, she could not be considered to be a normal subject. Be-

cause of these circumstances the values obtained on this person have questionable significance but are nevertheless included in the figure.

DISCUSSION

These results are interpreted as signifying that the acute rise in 17-ketosteroids after the administration of testosterone is a manifestation of the conversion of this steroid to these urinary products. Variations in the endogenous manufacture of precursor hormones could not be accurately evaluated but it is believed that these acute experiments obviated the effects of these fluctuations. Although the administration of a single dose of testosterone may influence the rate of formation of these precursors to some extent, the differences observed in the two phases of the menstrual cycle appeared to be greater than could be accounted for entirely on this basis. At least the administration of a single large dose (100 mg.) of methyltestosterone, an androgen which is not metabolized to the urinary 17-ketosteroids, did not influence the relatively constant excretion of 17-ketosteroids in a normal man.

The results show that normal women, like normal men, vary markedly in the extent to which they excrete the 17-ketosteroid metabolites formed from testosterone. A more striking finding is that these normal women consistently converted a greater portion of the administered steroid to 17-ketosteroids in the follicular than in the luteal phase. Without knowledge of the precise nature or intermediary metabolism of the adrenal cortical precursors of the 17-ketosteroids it is not possible to interpret these results directly in terms of adrenal cortical function. On the other hand if the metabolic pathway taken by testosterone is in part analogous to that taken by the adrenal steroids which are the precursors of the urinary 17-ketosteroids, then these studies suggest the possibility that the activity of the adrenal cortices of normal women may indeed fluctuate in a regular manner. Thus, if it is assumed that these adrenal steroids are, like testosterone, also metabolized to 17-ketosteroids to a greater extent during the very early part of the menstrual cycle, it then follows that the amount of these precursors produced by the adrenal must have been less during this period than during the later phases. Otherwise, a striking difference in the 17-ketosteroid excretion between the two phases of the cycle would be normally manifest, instead of the monotonous regularity observed by other investigators as well as by ourselves.

Since these results obviously are concerned only incidentally with adrenal cortical function, it is necessary to consider other available evidence which pertains to the influence of ovarian activity on these functions. There is a considerable amount of experimental data to indicate that the size and possibly even the function (13) of normal adrenal cortices can be in-

fluenced by ovarian activity. In rats, ovariectomy causes a diminution (1) whereas administered estrogens cause an increase (2) in the size of the adrenal cortices. These effects appear to be mediated through the pituitary in some manner (4). Further, in the rat it has been well established that the adrenal cortex enlarges significantly during estrus (3, 12). Although it is likely that a direct correlation exists between adrenal cortical size and adrenal activity, it is not easy, even in the experimental animal, to prove this correlation without actual measurements of the quantities of circulating hormones (14, 15).

Data on the influence of ovarian activity on adrenal cortical function in women are more inconclusive than in the experimental animal. Davis and Hulit (16) observed lower eosinophil counts at the time of ovulation and in the luteal phase than in the follicular phase of the cycle; the lowest counts were observed at about the time of ovulation. Thus, their studies and our own suggest greater adrenal cortical activity in the luteal than in the follicular phase. It is possible that we should have observed even greater differences in the metabolism of testosterone had the studies been made at the time of ovulation instead of later in the cycle. However, on the basis of clinical observations in different states, conflicting interpretations exist on the influence of estrogens on adrenal function, *e.g.*, that lack of estrogen probably increases adrenal cortical activity (17, 18), and that the administration of estrogen increases adrenal activity (19). Yet, it has been demonstrated that the administration of estrogens to men and women under a variety of circumstances and in different disorders diminishes the excretion of 17-ketosteroids (8, 20, 21). The effect of progesterone on adrenal activity in man has not been clearly defined (15). Therefore, it must be concluded that, as yet, there is no proof that the adrenal cortices of normal women undergo cyclic fluctuations in activity. The investigations herein reported suggest such fluctuations only if it is assumed that the metabolic pathway of testosterone is at least similar to that of those cortical hormones which are precursors of the 17-ketosteroids.

Since the results of this study show an altered rate of metabolism of a steroid which is a precursor of the urinary 17-ketosteroids, during different phases of the menstrual cycle, it is pertinent to consider the findings of Koets (10) on hirsute women. He found that the excretion of 17-ketosteroids by women with "idiopathic" hirsutism did fluctuate with the menstrual cycle. Although the peak excretion appeared at the time of ovulation, the excretion of 17-ketosteroids was generally higher, when determined in the later, than in the very early phases of the cycle. In the light of the experiments here reported it would seem possible that the basic defect in these women does not reside primarily in the adrenal cortices but rather in the manner in which their peripheral tissue (end organs?) handle

or metabolize precursor hormones. Since the data on normal women show a regular but varying ability to transform testosterone to 17-ketosteroids, these hirsute women may not possess such a regulatory mechanism for the metabolism of androgenic substances. If so, it is possible that even other so-called "endocrine" disorders have their genesis in similar manner. Indeed, if the adrenal cortices of normal women undergo cyclic fluctuations in function, while the excretion of 17-ketosteroids remains relatively constant, it is likely that changing factors residing in those peripheral tissues involved in the metabolism of precursor hormones, condition and control the rates of these metabolic reactions. Whether the action of ovarian hormones (estrogens or progesterone) or of other products causes these variations in tissue activity is unknown. However, Koets suggested another possibility to account for the fluctuant excretion of 17-ketosteroids in the hirsute women, *viz.*, that the adrenal cortices of these women are abnormally "sensitive" to ovarian influences. Of necessity this hypothesis would imply a defect in the regulation of the formation and release of adrenocorticotropin, as this is influenced by adrenal cortical and ovarian hormones. This possibility can not be easily investigated and the studies herein reported indicate another possible mechanism.

SUMMARY

Normal women metabolize more of the 19-carbon steroid, testosterone, to urinary 17-ketosteroid in the follicular than in the luteal phase of their menstrual cycle. Since the 17-ketosteroid excretion of normal women remains relatively constant throughout the menstrual cycle, it is possible that the adrenal cortices of normal women do fluctuate in activity but that regulatory mechanisms concerned with the metabolism of precursor hormones are such that a constant excretion of urinary steroids results. It has been reported (10) that the excretion of 17-ketosteroids of hirsute women varies in a regular manner during the menstrual cycle. If the studies herein reported truly represent different rates of metabolism of a steroid hormone by normal peripheral tissue, it would seem possible that the fundamental defect in these hirsute women may reside in the manner in which precursor hormones are metabolized.

Acknowledgment

The authors greatly appreciate the technical assistance of Mr. Leonard Cook and Mr. Pasquale J. Costa. Without their help this work could not have been carried out.

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THE IODINE VAPOR STAINED VAGINAL SMEAR: ITS USE IN OFFICE PRACTICE

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ATTENTION was drawn to the usefulness of the human vaginal smear in the clinical investigation of endocrine problems by Papanicolaou (1) in 1933. He described the cyclic changes that may be observed in the vaginal smear during the normal menstrual cycle. In a later paper (2) he reported the atrophic changes in the vaginal smears of menopausal patients and the reversal of these changes under estrogen therapy. Subsequently, various other investigators have used the vaginal smear to evaluate the effect of therapy in the menopause (3, 4), in amenorrhea (5), and in gonorrheal vaginitis in children (6); to assay and compare various estrogenic preparations and the methods of administering them (7, 8, 9); to attempt to detect ovulation (10, 11), and as a diagnostic and prognostic aid in abortion (12, 13).

In view of the complexity of the staining technic described by Papanicolaou, a number of modifications and other stains have been introduced. One of the simplest and easiest to interpret is the iodine vapor stain reported by Mack in 1942 (14, 15). The vaginal smear is suspended over Lugol's solution. The iodine vapor emanating from the solution stains the cells of a fully estrogenized smear a rich golden brown. The smear may then be graded on the basis of the amount of glycogen in the cells and the size, shape and number of the cells. The technic is easily carried out in office practice without the need for laboratory facilities, since all that is required is a dish of Lugol's solution, clean glass slides and a microscope. The slides may be prepared and inspected in a few minutes' time while the patient is in the physician's office.

Willson and Goforth (16) have reviewed some of the evidence that estrogens are responsible for glycogen deposition in the vaginal epithelium. They pointed out that vaginal glycogen is present in the newborn infant and in sexually mature females. It is absent in childhood and tends to be absent or diminished in the postmenopausal period. Glycogen appears in children and increases in menopausal females under treatment. Glycogen is known to accumulate in the cells and secretions of the endometrium during the menstrual cycle (17). This effect, is apparently estrogenic

Received for publication September 25, 1950.

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since Boettiger (18) showed that estrogens stimulate glycogen deposition in the uterus of the castrate rat, the glycogen level remaining high as long as estrogen is present. Progesterone has no effect on uterine glycogen. It is perhaps reasonable to assume that the deposition of glycogen in the endometrium resulting from estrogenic stimulation is paralleled by a similar effect in the vaginal epithelium.

Salmon, Walter and Geist (19) have shown that the administration of adequate amounts of testosterone to a normal female will result in an atrophic smear with absent glycogen. Willson and Goforth (16) found that the amount of glycogen in the vaginal smear bore no relation to the dietary intake of carbohydrate.

The present study was carried out to evaluate the usefulness and limitations of the iodine vapor stained vaginal smear in office practice. Smears were obtained from approximately 500 female patients during a period of a year and a half in the medical department of the Lahey Clinic. The group was selected to consist of interesting endocrine problems, menopausal and postmenopausal women, and many normal women of childbearing age.

TECHNIC AND CLASSIFICATION OF SMEARS

The technic of preparing and staining the smears was essentially that described by Mack (14, 15). A cotton applicator moistened with normal saline solution is inserted into the vagina and twirled against the wall. The applicator is then rolled evenly along the surface of a clean glass slide, leaving a thin film. After several minutes to allow for drying, the slide is placed face down over a dish of fresh Lugol's solution for five minutes. The slide is then ready for examination. (The Lugol's solution should be kept covered when not in use and should be changed frequently, as it gradually losses its potency.) The stained slide will fade in time but may be restained as much as a year or more later.

The smears were classified Grade 1 to 4, according to criteria similar to those of Mack. The classification is somewhat arbitrary but it was arranged so that the smears of the majority of sexually mature females fell into Grade 4, the most atrophic smears into Grade 1, and the intermediate ones into Grades 2 and 3.

The criteria were:

Grade 1. Complete glycopenia (less than 1 per cent of cells stained). Small cells of varying size and shape, many of them round; there may be almost complete absence of cells.

Grade 2. Cells show glycogen in irregular deposits at margins or scattered in cytoplasm. Scattered cells uniformly but lightly stained (less than 20 per cent). Cells mostly small.

Grade 3. Cells stained diffusely but lightly. Cells larger and more

regular. Many noniodophilic yellow cells. Scattered cells deeply stained (less than 25 per cent).

Grade 4. Well stained golden-brown cells. Some cells unstained but all are large, flat and with small unstained nuclei.

RESULTS

Normal menstruation. Vaginal smears were obtained from 39 patients with regular menstrual cycles. The smears were characterized by large, flat, golden-brown cells with pyknotic nuclei. Smears of this type were classified as Grade 4. The number of unstained cells varied widely. During the few days preceding the onset of the menses, a fairly marked glycopenia was frequently found. The appearance of the smears did not change significantly during the phases of the menstrual cycle.

Menstrual disorders. Sixteen cases of menorrhagia, metrorrhagia and oligomenorrhea were included in the study. The smears were distributed as follows: twelve Grade 4, three Grade 3, and one Grade 2. The type of the menstrual disorder could not be correlated with the grade of the smear.

Menopause. The 69 patients included in this group were all at least 40 years of age, had had no menstrual bleeding and had received no hormonal therapy during the preceding six months. No stigmata of any endocrine disease were present. Irregular cells and relative glycopenia were characteristic of the smears in this group. There were nine Grade 4, thirty Grade 3, twenty-one Grade 2, and nine Grade 1 smears. Grade 4 smears were found as long as twelve years after the cessation of menses and Grade 3 smears were seen twenty years later. The presence of a moderate amount of glycogen was the usual finding in a postmenopausal patient.

The smears from 6 patients who had previously undergone bilateral oophorectomy were distributed as follows: one Grade 4, three Grade 3, and two Grade 2. The smears of 2 patients in whom the menopause was induced by roentgen rays, were Grade 4 and Grade 3.

Thirty-three of the 69 patients were aware of hot flashes. In patients with smears of Grades 2, 3 and 4, no correlation between the grade of the smear and the presence or severity of hot flashes was apparent. On the other hand, hot flashes were absent in 8 of the 9 patients with Grade 1 smears and infrequent in the remaining one.

Acromegaly. The data from 3 cases of acromegaly, 2 of them complicated by hyperthyroidism, are presented in Table 1. The smears varied from Grade 1 to Grade 4.

Anorexia nervosa. The diagnosis of anorexia nervosa was considered definite in 2 patients, 15 and 25 years old. In each there was a history of anorexia, marked weight loss and secondary amenorrhea. The results of the water test for adrenal function (Robinson-Power-Kepler test) were

normal, the total eosinophil counts were low (6 per cubic millimeter in each) and the twenty-four-hour excretion of 17-ketosteroids was 18.0 and 6.8 mg. in the 2 cases. In the latter case the amount of follicle-stimulating hormone in the urine was within normal limits. The vaginal smear in the 15-year-old girl at first was Grade 3, but during a six-week period changed to Grade 1 and remained so during five months of observation. The smear obtained from the 25-year-old patient was Grade 2. The atrophic smears of both these patients contained a moderate number of small round cells with large nuclei. The smears in both cases became fully glycogenized and menses were induced when estrogen therapy was instituted.

The diagnosis of anorexia nervosa was never definitely settled in another patient, a 16-year-old girl with primary amenorrhea and malnutrition. The smear in this case was Grade 3.

Data from these 3 cases are included in Table 1.

Chromophobe adenoma. Twelve patients with chromophobe adenomas of the pituitary were included in the study (Table 1). Diagnosis was based on the development of amenorrhea and the presence, by roentgenologic examination, of a definitely enlarged sella turcica. Confirmation was added in some cases by tests showing impaired adrenal and thyroid function. The diagnosis in 1 case had been verified at operation. The smears in 5 of the patients were well glycogenized and were classified Grade 4, although several of them appeared slightly atypical, with moderate numbers of small unstained cells present. The smears were relatively glycopenic in the remaining 7 cases. Small round cells were noted in several.

Menses were induced repeatedly in 2 of the Grade 4 cases by the buccal administration and withdrawal of Lutocylol.[1] The attempt failed in a third case.

Cushing's syndrome. Four cases of Cushing's syndrome are summarized in Table 1. These were all classic cases. In 2 patients with Grade 4 smears menses were induced with buccal Lutocylol therapy. Two other patients, one aged 31 and the other aged 52 years, had atrophic Grade 2 smears.

Diabetes insipidus. One patient with diabetes insipidus complicated by amenorrhea of eighteen months' duration had a well glycogenized Grade 4 smear. An atrophic smear was found in a 67-year-old woman with moderately severe diabetes insipidus. She had had an operation eight months previously for a tumor of Rathke's pouch (Table 1).

Hirsutism. Two hirsute females with normal menses had Grade 4 smears (Table 1).

Myxedema. Seven cases of spontaneous myxedema were studied and are

[1] Anhydrohydroxyprogesterone, supplied by courtesy of Ciba Pharmaceutical Products, Inc. The dosage used was 30 to 75 mg. daily for five to seven days.

TABLE 1. VAGINAL SMEARS IN VARIOUS ENDOCRINOPATHIES

Diagnosis	Number of cases	Smear, grade	Age, years	Remarks
Acromegaly	1	4	32	Menses induced with buccal Lutocylol.
	1	3	34	Also hyperthyroidism; x-ray treatment to pituitary ten months previously.
	1	1	58	Also hyperthyroidism; x-ray menopause at age 35.
Anorexia nervosa	1	3-2-1	15	Smear changed from Grade 3 to Grade 1 in six weeks without any therapy. No bleeding after Lutocylol treatment. Weight loss 60 pounds. Amenorrhea for nine months.
	1	2	25	Weight loss, 45 pounds. Amenorrhea for five years.
	1	3	16	Diagnosis questionable. Amenorrhea primary.
Chromophobe adenoma of pituitary	5	4	22–39	Menses induced in 2 cases with buccal Lutocylol; failure in 1. Previous x-ray treatment, 1 case. Surgical castration ten years previously, 1 case (age 29); hot flashes.
	6	3	28–43	Previous x-ray treatment, 1 case. Previous x-ray treatment and radical removal, 1 case. Surgical castration three years previously, 1 case. Lutocylol, given bucally, failed to induce menses, 1 case.
	1	2	21	
Cushing's syndrome	2	4	26–28	Previous subtotal adrenal resection, 1 case. Menses induced in both cases with buccal Lutocylol.
	2	2	31–52	
Diabetes insipidus	1	4	31	Amenorrhea, eighteen months.
	1	2	67	Moderate diabetes insipidus. Operation for tumor of Rathke's pouch six months previously.
Hirsutism	2	4	23–32	Menses present.

TABLE 1—(*continued*)

Diagnosis	Number of cases	Smear, grade	Age, years	Remarks
Myxedema	4	4	13–45	Thyroid therapy, 3 cases; 1 pregnancy.
	1	3	58	Ovaries removed eight years previously. No thyroid therapy.
	2	1	44–47	Menopause fifteen years previously (age 57). Secondary hypopituitarism; thyroid therapy, 1 case.
Ovarian agenesis	1	3	18	Estrogen treatment six months previously.
	2	2	26–27	Recent treatment, 1 case.
	2	1	14–16	Smears Grade 4, after treatment.
Simmonds' disease	3	1	36–54	Onset in 2 cases after postpartum hemorrhage (Sheehan's disease).

included in Table 1. Normal smears were found in 4 patients who ranged in age from 13 to 45 years.

Varying degrees of glycopenia were observed in 2 patients who were postmenopausal and in 1 with hypopituitarism, apparently secondary to the myxedema. The grade of the vaginal smear appeared to be unrelated to the presence of myxedema or to the use of thyroid therapy.

Ovarian agenesis. The data from 5 cases of ovarian agenesis are presented in Table 1. The diagnosis in these cases was based on short stature, retarded bone age, failure of secondary sexual development, amenorrhea and the presence of increased amounts of follicle-stimulating hormone in the urine. The smears were characteristically atrophic. Small round unstained cells with large nuclei were frequently seen. These cells occurred commonly in only one other condition, Simmonds' disease; but were also seen in chromophobe adenoma of the pituitary and in anorexia nervosa.

Simmonds' disease. Three cases of Simmonds' disease were studied. The diagnosis was based on complete loss of axillary and pubic hair, a positive response to the water test, a twenty-four-hour urinary 17-ketosteroid excretion of less than 3.0 mg. and a basal metabolic rate of −25 per cent or lower. Data from these cases are listed in Table 1. The vaginal smears were characteristic. They showed virtually complete glycopenia, a paucity of cells and the frequent occurrence of small round cells with large nuclei.

Amenorrhea. Seventeen additional patients not included in any of the above endocrinopathic entities were also studied because of amenorrhea

(Table 2). The vaginal smears varied from well glycogenized to glycopenic, there being eight Grade 4, six Grade 3, and three Grade 2 cases. There appeared to be a correlation between the grade of vaginal smears and the results of endometrial biopsies. Biopsies in three Grade 4 cases revealed normal proliferative endometrium in 2, but no endometrium was obtained

TABLE 2. VAGINAL SMEAR IN AMENORRHEA

Age, years	Smear, grade	Duration of amenorrhea	Remarks
24	4	13 months	Biopsy—proliferative endometrium. Spontaneous periods recurred later.
23	4	22 months	Post partum—lactating still, but did not nurse baby. Biopsy—proliferative endometrium.
32	4	26 months	Post partum—no endometrium, on biopsy. Urinary FSH not increased.
21	4	5 months	Two to 3 periods a year, in past. Regular periods initiated with cyclic Lutocylol therapy alone.
33	4	14 months	Hot flashes. Two periods followed buccal Lutocylol; then failed twice.
38	4	16 months	Patient feels as if period imminent, but none occurs.
27	4	30 months	FSH normal. Skull not enlarged. Normal development. Previous regular periods.
28	4	6 months	Sella not enlarged.
30	4	12 months	No hot flashes.
27	3	18 months	Endometrial biopsy specimen insufficient for diagnosis. FSH normal. No bleeding after Lutocylol treatment.
23	3	17 months	Hot flashes for three to four months.
33	3	30 months	FSH normal. No hot flashes.
32	3	Always	FSH normal. Some recent therapy. Infantile cervix; uterus not felt.
24	3	12 months	Uterine hypoplasia. Endometrial biopsy specimen insufficient for diagnosis.
34	2	Periods few and scant	Later, smear Grade 3 on Lutocylol therapy. Period occurred. Eight days later, smear Grade 4.
19	2	Always	Height 61 inches. No increase in FSH; 17-ketosteroids, 14.3 mg. per 24 hrs.
25	2	5 years	Platybasia. FSH normal.

in the third. Biopsy specimens in two Grade 3 cases produced insufficient material for diagnosis.

Cyclic administration of Lutocylol alone by the buccal route in 2 patients with Grade 4 smears regularly resulted in menstrual bleeding on withdrawal of the drug in 1 case. The other patient responded twice but no bleeding occurred on two further trials. This therapy was without effect in another patient with a Grade 3 smear. A menstrual period was produced in a patient with a Grade 2 smear, however, following which the

smear was found to be Grade 4. Spontaneous recovery of ovarian function may have occurred simultaneously with the treatment.

Effect of estrogenic therapy. Many menopausal and other hormonally deficient patients were treated with the usual therapeutic doses of both natural and synthetic estrogenic substances. Grade 4 smears resulted in these cases.

In addition, a number of patients with endometriosis and a few with other conditions were given massive doses of estrogenic compounds. The vaginal smears at the onset of therapy were Grade 4 in many of these cases and no change occurred. For example, one 45-year-old woman with a mass in the cul-de-sac was treated with 1,200 mg. of diethylstilbestrol orally in a sixty-day period, at the end of which the smear was Grade 4 and the mass was not palpable. The smear was also Grade 4 in a 46-year-old woman who had received 3,600 mg. of dienestrol orally during six months. A mass in her breast disappeared. On the other hand, therapy resulted in moderately atrophic Grade 3 smears in some cases. This was true of a 23-year-old patient with verified endometriosis who received 750 mg. of dienestrol orally during two months' time, and of a 36-year-old woman given 3,025 mg. of diethylstilbestrol orally in twenty months. The morphology of the cells in these smears was similar to that of well glycogenized smears, but the staining was faint.

DISCUSSION

The classification of vaginal smears used in this investigation was an arbitrary one. That the grade of the smear probably does give a rough indication of the estrogenic titer of the patient is indicated by the fact that women with normal menstrual cycles with very few exceptions had Grade 4 smears, in contrast to the patients with Simmonds' disease who uniformly had Grade 1 smears. One would expect normal amounts of estrogen to be present in the former group but only a bare minimum, if any, in the latter group. Also, the fact that menses could be induced with progesterone therapy alone in a number of amenorrheic patients with Grade 4 smears would indicate that nearly normal estrogen titers must have been present in these patients.

Further evidence of a correlation between the grade of the vaginal smear and the estrogenic titer was provided by the results of a series of endometrial biopsies in patients with amenorrhea, which showed normal proliferative endometrium in two of three Grade 4 cases, and atrophic endometrium in two Grade 3 cases.[2]

[2] In a preliminary study of iodine vapor stained urethral smears in males, the amount of glycogen present in 1 patient with cirrhosis of the liver and in another patient who received large amounts of stilbestrol, approached that observed in normal females, whereas only small amounts were found in normal males.

The occurrence of relatively glycopenic smears in patients who had received large amounts of estrogens was surprising. Ayre and Ayre (20) reported glycopenic smears in 6 patients who received large amounts of stilbestrol dipropionate. In these same cases, smears prepared with Papanicolaou's stain showed large numbers of superficial squamous cells, most of which were cornified, indicating hyperestrinism. Apparently highly cornified cells contain reduced amounts of glycogen or for some other reason do not take up iodine vapor well. Consequently, the usefulness of the iodine vapor stained vaginal smear is limited to those conditions in which normal or reduced amounts of estrogen are present. It may be misleading in hyperestrinism.

The iodine vapor stained vaginal smear did not prove to be useful in interpreting the phases of the normal menstrual cycle or in managing abnormal menstrual bleeding.

The vaginal smear in the menopause was characteristically moderately glycopenic but in individual cases ranged from completely atrophic to well glycogenized. Glycogen tended to persist for many years after the natural menopause and also in surgically and roentgen-ray induced castrates. Persistently "positive" vaginal smears have been noted by others during the menopause and after castration (4). One must assume that this indicates the continued production of estrogens. Frank, Goldberger and Salmon (21) have, in fact, demonstrated significant estrogenic excretion by patients who have had both ovaries removed. The adrenal gland is a possible source of this estrogen. It is interesting in this connection that a fivefold to eightfold increase in estrogen excretion following surgical procedures in 3 postmenopausal females, 1 a surgical castrate, was demonstrated by Smith and Smith (22), apparently as a part of the alarm reaction.

The postmenopausal patients with very atrophic smears seldom experienced hot flashes. Otherwise, there was no correlation between the grade of the vaginal smear and the presence or absence of hot flashes. Failure to find a relationship has also been reported by others (4, 21).

The iodine stained vaginal smear in Simmonds' disease was characterized by nearly complete glycopenia, a paucity of cells and the presence of small round cells with large nuclei. Similar smears were obtained in cases of ovarian agenesis but they could usually be distinguished by the presence of greater amounts of glycogen. A series of smears in 1 case of anorexia nervosa was at first fairly well glycogenized but later resembled those obtained in Simmonds' disease. A smear obtained from a second patient with anorexia nervosa was also moderately atrophic, with some small round cells present.

The vaginal smear was not diagnostic in acromegaly, chromophobe adenoma of the pituitary, Cushing's syndrome, myxedema or diabetes

insipidus, and was not unusual in hirsutism. It was of some aid in studying a number of other patients with amenorrhea. In a variety of cases the vaginal smear proved to be an aid in management, in that the unexpected presence of well estrogenized smears led to the production of menstrual cycles by the periodic use of progesterone therapy alone. Withdrawal bleeding following the administration of progesterone without estrogen priming has also been reported recently by others (23, 24).

SUMMARY

The iodine vapor stained vaginal smear proved to be useful as a rough index of estrogenic titer in patients with normal or reduced estrogen levels. It may be misleading in hyperestrinism.

The vaginal smear was not helpful in interpreting the phases of the normal menstrual cycle or in evaluating abnormal menstrual bleeding.

The vaginal smear was usually characteristic in the menopause. Moderate amounts of glycogen tended to persist for many years after the natural menopause and also in surgically and roentgen-ray induced castrates, indicating a nonovarian locus of estrogen production.

Fairly characteristic vaginal smears were obtained in cases of Simmonds' disease and ovarian agenesis. The vaginal smear, however, was not a reliable criterion for distinguishing between Simmonds' disease and anorexia nervosa.

Vaginal smears are described in cases of acromegaly, chromophobe adenoma of the pituitary, Cushing's syndrome, diabetes insipidus, myxedema and hirsutism.

In patients with amenorrhea, menstrual bleeding could usually be produced with anhydrohydroxyprogesterone alone when the vaginal smear was well glycogenized, thus eliminating the preliminary use of estrogens. On the other hand, in patients with atrophic smears, bleeding usually did not occur.

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Letter to the Editor

NOMENCLATURE IN CHEMICAL ADRENAL STEROID ASSAYS

To the Editor:

The increasing use of chemical methods for the quantitative determination of substances of adrenal cortical origin has been attended by wide, confusing variations in terminology.

Certain usages, such as "11-oxycorticosteroid" and its even more unfortunate twin "11-desoxycorticosteroid" seem pretentious and are inexact. In most such instances the assumption is made that a benzene-water partition of lipid fractions provides a quantitative separation of steroids with and without oxygen atoms at C 11. This belief is unjustified (Heard, R. D. H., and Sobel, H.: *J. Biol. Chem.* **165**: 687, 1946). Those who recognize that it is only partly true often indicate it by quotation marks or explanatory footnotes. These terminologic subterfuges confuse the casual reader; nor can they be justified on grounds of necessity or convenience, since there are adequate terms in current use.

From the chemical point of view, what is usually measured is a lipid (L) fraction; and the measurement is made in terms of formaldehyde production (F) or of reducing power (R). We have then the terms "formaldehydogenic lipid" and "reducing lipid," often abbreviated as FL and RL. These, although precise, are not informing to the clinician.

The assumption that most of the substances measured are of adrenal cortical origin is probably justified. Consequently, we and others have used the term "corticosteroid." But it also is inexact, since substances which are not steroids and which are not of adrenal cortical origin contribute in some part to the material measured. The term "corticoid" covers this fact. It implies that what is measured is something which has some of the properties of an adrenal steroid. Its degree of imprecision corresponds well with that of methods in current use. At the same time it is clinically informing. Thus, the terms "formaldehydogenic" (F) or "reducing" (R) "corticoids" seem adequate.

Our method (Corcoran, A. C., and Page, I. H.: *J. Lab. & Clin. Med.* **33**: 1326, 1948) for the measurement of "formaldehydogenic corticoid" does not involve a benzene-water partitition, because a considerable experience convinced us that it added nothing towards our purpose. What is to be done when the method used does involve this procedure or an analogous

step? The terms "relatively water-soluble" and "relatively water-insoluble" fit the situation. They are more cumbersome and less attractive than the prefixes "11-oxy" and "11-desoxy," but they connote a fact. The "oxy" prefixes should be reserved until the time when clinical methods are developed which will specifically denote the position of the oxygen atom.

Consequently, may I suggest that the Editorial Board of the Association for the Study of Internal Secretions consider recommending a similar system of usage in their publications? Much confusion would be obviated and the example would soon be widely followed.

A. C. CORCORAN, M.D.

Cleveland Clinic,
Cleveland, Ohio.

The Association for the Study of Internal Secretions

THE 1951 ANNUAL MEETING

The Thirty-third Annual Meeting of the Association for the Study of Internal Secretions will be held at the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey. The Scientific Sessions have been extended to three days, Thursday, Friday, and Saturday, June 7-8-9.

The Local Chairman of Arrangements is Dr. Matthew Molitch.

Due to the advance in the date of our meeting, Chalfonte-Haddon Hall Hotel will be unable to accommodate all of our members by reason of a previous commitment. The Colton Manor, on Pennsylvania Avenue near Haddon Hall, is an excellent hotel, which has been recently refurnished, and is holding one hundred rooms for our members on the "first come, first served" basis. The Hotel Strand, at Pennsylvania Avenue and the Boardwalk, has also promised to supply a limited number of rooms. It is important that reservations be made immediately if you are to avoid disappointment.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms, in which the session will be held, will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 P.M. in the Rutland Room, preceded by cocktails at 6:30 P.M. in the adjoining Benjamin West Room.

(For details about the type of program, *see* Dr. E. H. Rynearson's Letter to the Editor on page 1355 of the October 1950 issue, and Dr. Jane A. Russell's Letter to the Editor on page 229 of the February 1951 issue.)

Fourth Annual Postgraduate Assembly in Endocrinology Including Diabetes

Sponsored by

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

and

THE AMERICAN DIABETES ASSOCIATION

SEATTLE, WASHINGTON OLYMPIC HOTEL JULY 2–7, 1951

The faculty will consist of prominent researchers and clinicians in the field of endocrinology and metabolic disorders.

The course will be a practical one of interest and value to the specialist and those in general practice. The program will consist of lectures, clinics, and demonstrations. Ample time will be given to questions and answers at the end of each session, and registrants are encouraged to contact members of the faculty for individual discussions.

The Olympic, one of Seattle's most delightful hotels, offers special convention rates to members of this assembly. This is an unusual opportunity for you and your family to enjoy a pleasant vacation in the beautiful Pacific Northwest and for you to participate in a highly instructive program of the latest advances in endocrinology and metabolism.

A fee of $75 will be charged for the entire course and the attendance will be limited to 100. REGISTRATION WILL BE IN THE ORDER OF CHECKS RECEIVED AND WILL CLOSE ON JUNE 4, 1951. SHOULD THERE BE AN INSUFFICIENT NUMBER OF APPLICANTS TO FILL THE COURSE, THE REGISTRATION FEE WILL BE REFUNDED IMMEDIATELY IN ITS FULL AMOUNT.

Please forward application on your letterhead, together with check payable to The Association for the Study of Internal Secretions, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma, *before June 4, 1951*. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Olympic Hotel, Seattle, Washington, and the hotel advised that you are attending this Postgraduate Assembly.

The American Goiter Association

THE 1951 ANNUAL MEETING

THE 1951 annual meeting of the American Goiter Association will be held in Columbus, Ohio, on May 24, 25, and 26. The Deshler-Wallick Hotel, Broad and High Streets, will be the headquarters and every effort will be made by the hotel officials to make the meeting a success and the visit a very pleasant one. It would be well to see that reservations are made now.

The president, Dr. T. C. Davison, and the chairman of the Local Arrangements Committee, Dr. George Curtis, are planning an excellent meeting which will consist of papers dealing with goiter and other diseases of the thyroid gland, dry clinics and demonstrations. It is hoped that there will be a large attendance of members.

GEORGE C. SHIVERS, M.D.,
Corresponding Secretary,
Colorado Springs, Colo.

PRELIMINARY PROGRAM

Thursday—May 24

9:00 a.m.—General Session

Drs. Joseph L. and Cornelius DeCourcy—Etiology of Goiter.

Drs. Curtis and Fertman—Foods and the Genesis of Goiter.

Drs. Dobyns and Lennon—The Heterotransplantability of Human Thyroid Tumors (Benign and Malignant) to the Anterior Chamber of the Guinea Pig's Eye.

Drs. Lambert, Underdahl and Beckett—A Study of the Ankle Jerk in Myxedema.

SYMPOSIUM ON IODINE METABOLISM

Drs. Wyngaarden, Dutoit, Stanbury and Scheinberg—The Iodide Trapping Mechanism of the Thyroid Gland.

Drs. Danowski, Hill and Huff—Protein-Bound Iodine and Thyroxine in Rats Injected with Elemental Iodine or Inorganic Iodide.

Dr. Brownell—Analysis of Techniques for the Determination of Thyroid Function with Radioiodine.

Drs. Seed and Jaffe—Evaluation of the Diagnostic Use of Radioactive Iodine in Diseases of the Thyroid.

Drs. Salter, DeVisscher, McAdams and Rosenblum—Changes in Blood Iodine Fractions and Radioactivity Under Therapy.

2:00 p.m.—General Session

SYMPOSIUM ON DIAGNOSIS AND MEDICAL TREATMENT OF TOXIC GOITER

Richard L. Rapport—The Somnolent Metabolic Rate (SMR) as an Aid in the Differential Diagnosis of Thyroid Dysfunction.

Drs. Blackburn, Keating and Haines—Studies on Thiocyanate Myxedema.

Dr. Cogswell—Periodic Paralysis Associated with Hyperthyroidism.

Dr. Astwood—Use of Antithyroid Drugs During Pregnancy.

Drs. Maloof and Chapman—The Treatment of Thyrotoxicosis Manifested Chiefly by Cardiovascular Disturbances.

Drs. Skillern and McCullagh—Cases of Graves' Disease Resistant to Radioactive Iodine Therapy.

Dr. Bartels—Methyl Mercaptoimidazole—An Active Antithyroid Agent.

Drs. Goldsmith, Stanbury and Brownell—The Effect of Thyrotropic Hormone on Patients with Thyrotoxicosis Treated with 2-Methylmercaptoimidazole.

Dr. Thompson—The Present Status of the Treatment of Toxic Goiter.

Friday—May 25

9:00 a.m.—General Session

Van Meter Prize Essay.

SYMPOSIUM ON THYROIDITIS

Dr. Altemeier—Acute Pyogenic or Suppurative Thyroiditis.

Drs. Robbins and Trunnell—Effect of Thyroid-Stimulating Hormone in Acute Thyroiditis.

Drs. Goetsch and Kamner—Chronic Thyroiditis; Etiology and Pathogenesis.

Dr. Crile—Thyroiditis, with Special Reference to Classification and Aspiration Biopsy with the Silverman Needle.

Dr. McConahey—Radioiodine Studies in Thyroiditis.

Business Meeting. Executive Session.

2:00 p.m.—General Session

Presidential Address. Dr. T. C. Davison.

SYMPOSIUM ON SURGICAL ASPECTS OF GOITER

Dr. Woodman—The Open Approach for Arytenoidectomy.

Dr. Lahey—Some of the Complications of Thyroidectomy and Their Management.

Dr. Bartlett—Recurrent and Persistent Hyperthyroidism.

Dr. Hunt—Differential Diagnosis and Surgical Management of Deep Cervical and Intrathoracic Goiter.

Dr. Mahorner—Goiters in Guatemala.

Dr. Carraway—Intravenous Pentothal Oxygen Anesthesia and Thyroid Surgery.

Drs. Ramey, McKenney and Scott—Postoperative Parathyroid Deficiency Following Thyroidectomy.

Saturday—May 26

9:00 a.m.—General Session

SYMPOSIUM ON THYROID TUMORS

Drs. Money, Fitzgerald and Godwin—Radioautographic Studies of Experimentally Induced Thyroid Tumors.

Drs. Rall, Miller, Trunnell, Foster, Peacock and Rawson—The Increased Uptake of I^{131} by Metastatic Cancer of the Thyroid Induced by Thiouracil.

Dr. Beahrs—Nodular Goiter and Carcinoma of the Thyroid; Statistical Review of 381 Cases.

Dr. Winship—Carcinoma of the Thyroid in Children.

Drs. Ravdin and Horn—Carcinoma of the Thyroid in the Young.

Drs. Hendrick and Reed—Carcinoma of the Thyroid Gland.

Drs. Dobyns and Maloof—A Progress Report of the Treatment of Carcinoma of the Thyroid with Radioiodine.

Drs. Chesky, Dreese and Hellwig—Hürthle Cell Tumors of the Thyroid Gland. A Report of 25 Cases.

Drs. Rawson, Rall and Peacock—Indications and Limitations in the Treatment of Cancer of the Thyroid with Radioactive Iodine.

Laurentian Hormone Conference

THE Laurentian Hormone Conference of the A.A.A.S. will hold its 1951 meeting at Mont Tremblant Lodge, Mont Tremblant, Quebec, from September 9 to 14. The following program has been arranged:

I. Pituitary Hormones

Chemistry of corticotrophin
Drs. E. B. Astwood, M. S. Raben and R. W. Payne, The Boston Dispensary
Sunday evening, September 9

Purification of the pituitary adrenocorticotrophic hormone
Drs. C. J. O. R. Morris, M. L. Deaman and P. Morris, The London Hospital
Monday morning, September 10

Regulation of ACTH secretion
Dr. C. N. H. Long, Yale University School of Medicine
Monday morning, September 10

Aspects of pituitary-mammary gland relationships
Dr. S. J. Folley, University of Reading
Monday evening, September 10

II. Sex Cycles

The relationship of the nervous system to the release of gonadotrophin and the regulation of the sex cycle
Drs. J. E. Markee, John Everett and C. H. Sawyer, Duke University School of Medicine
Tuesday morning, September 11

Some physiological and medical aspects of the gonadal cycle of domestic animals
Dr. F. X. Gassner, Colorado Agricultural and Mechanical College
Tuesday morning, September 11

Endocrinology and related phenomena of the human menstrual cycle
Drs. G. V. and O. W. Smith, Free Hospital for Women
Tuesday evening, September 11

III. Aspects of Steroid Hormone Chemistry and Physiology

Studies on the biosynthesis of adrenal cortex hormones
Dr. W. J. Haines, The Upjohn Company
Wednesday morning, September 12

The role of the adrenal cortex in the regulation of stress-induced tissue reactions
Dr. T. F. Dougherty, University of Utah College of Medicine
Wednesday morning, September 12

A comparison of the histological changes induced by experimental hyperadrenocorticalism and inanition
Dr. B. L. Baker, University of Michigan Medical School
Thursday morning, September 13

Histamine, hormones and hypersensitivity
Dr. Bram Rose, Royal Victoria Hospital
Thursday morning, September 13

IV. HORMONES AND METABOLISM

Studies of the hormonal factors which regulate the mobilization of depot fat to the liver
Dr. Louis Levin, Columbia University College of Physicians and Surgeons
Thursday evening, September 13
The etiology of diabetes mellitus
Dr. I. A. Mirsky, The May Institute for Medical Research
Friday morning, September 14
Some clinical aspects of adrenal cortical and fluid metabolism
Dr. C. W. Lloyd, Syracuse University College of Medicine
Friday morning, September 14

Attendance at this Conference is limited by the accommodation available at the hotel, but the Committee on Arrangements invites applications for attendance from interested investigators and specialists in the hormone field. The Committee on Arrangements consists of R. W. Bates, R. D. H. Heard, A. D. Odell, E. C. Reifenstein, Jr., A. White and G. Pincus, Chairman. Application blanks for attendance at the Conference may be obtained from the Chairman at 222 Maple Avenue, Shrewsbury, Massachusetts. Applications to be considered by the Committee must be received by *June 15, 1951.*

PHYSICIANS' ART SHOW AT AMERICAN MEDICAL ASSOCIATION MEETING ATLANTIC CITY

The American Physicians Art Association will have an art exhibit, as usual, during the A.M.A. convention at Atlantic City, N. J., June 11 to 15, 1951, inclusive. Any physician in the United States, Canada and Hawaii desiring to participate in this show should communicate with the secretary for particulars.

J. Henry Helser & Co., Inc., Investment Managers with offices on the Pacific Coast, are the new sponsors of the American Physicians Art Association and will award 200 trophies besides a special Helser Trophy—a large decorative cup depicting Yankee Ingenuity. This cup is to be awarded for art work done in any medium. Also the large Popularity Trophy will be awarded to the owner of the art piece receiving the most popular votes during the A.M.A. convention. Over 4000 members of the American Physicians Art Association will receive shortly, entry blanks, shipping labels and rules about this fourteenth art exhibition.

The Annual Art Banquet will be held Tuesday evening, June 12, at the Marlborough-Blenheim Hotel, Atlantic City, N. J.

F. H. Redewill, M.D., Sect'y
American Physicians Art Association
760 Market Street
San Francisco 2, California

SECOND INTERNATIONAL GERONTOLOGICAL CONGRESS

The Second International Gerontological Congress will be held at the Hotel Jefferson, St. Louis, Missouri, *September 9–14, inclusive, 1951.* It will be under the sponsorship of the International Association of Gerontological Societies, the Gerontological Society, Inc. and the American Geriatrics Society.

Section I of the program—Biology and Medicine—is directed by John Esben Kirk, Dept. of Gerontology, Washington University Medical School, 5600 Arsenal Street, St. Louis 9, Mo. Section II—Sociology, Psychology, Education and Religion—Dr. Robert J. Havighurst, Committee on Human Development, University of Chicago, Chicago 37, Ill. Section III—Economics and Welfare—Professor Edwin E. Witte, Dept. of Economics, University of Wisconsin, Madison, Wis. Section IV—Medical Services, Hygiene and Housing—Dr. Joseph W. Mountin, Associate Chief, Bureau of Medical Services, U. S. Public Health Service, Washington, D. C. Program Directors are assisted by Advisors.

Communications concerning papers should be sent to the Director concerned. The general plan is to have many short papers, several symposia, and sessions devoted to problem identification in each division. Dr. Albert I. Lansing, Dept. of Anatomy, Washington University Medical School, 660 South Kingshighway, St. Louis 10, Mo., is the Chairman of the Committee on Exhibits. *Titles of papers with abstracts of not more than 200 words must be received by the Program Directors before May 1 and descriptive summaries of exhibits by Dr. Lansing, likewise before May 1.*

The registration fee is $10.00. It is recommended that room reservations be made as soon as possible at the Jefferson Hotel, St. Louis 3, Mo.